Volume 29, Number 3, December, 1956

The Yale Journal of Biology & Medicine

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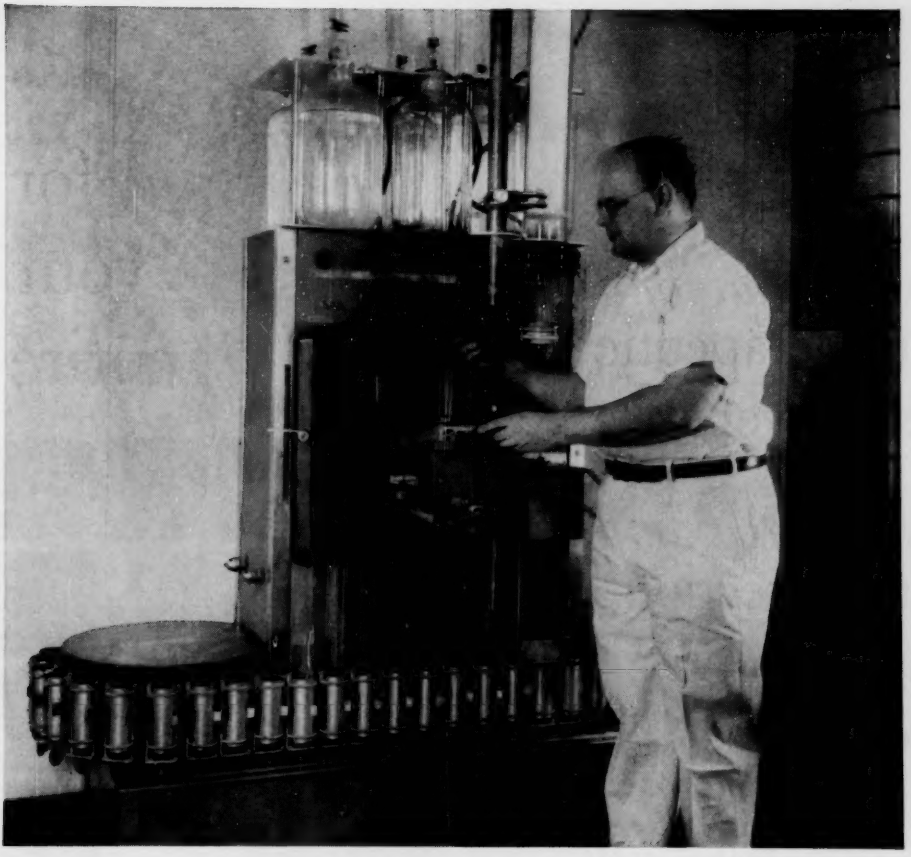

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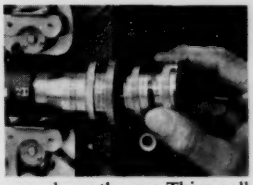

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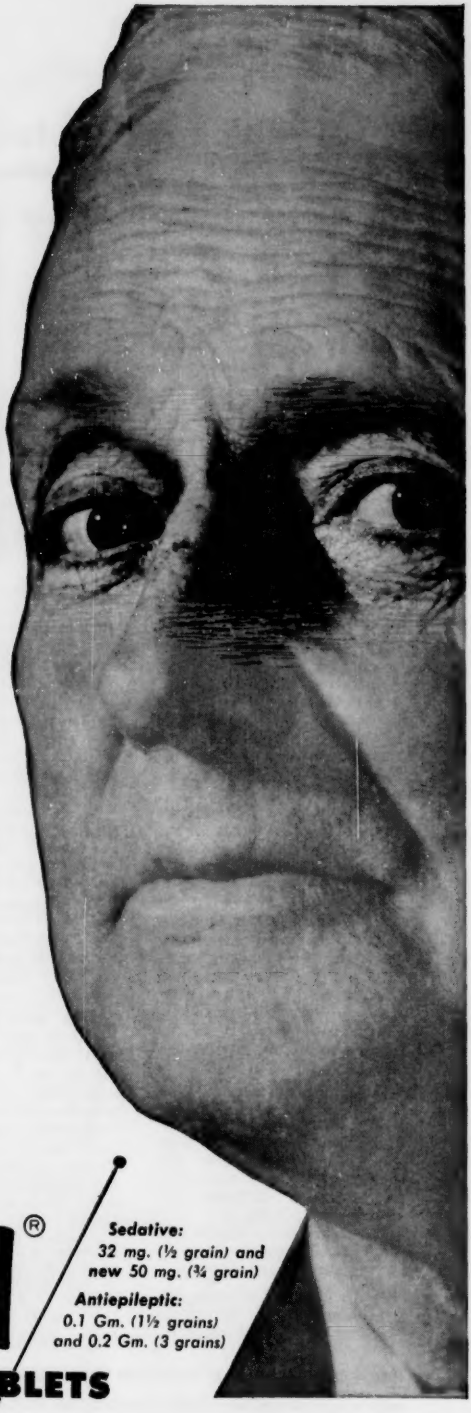

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edited by

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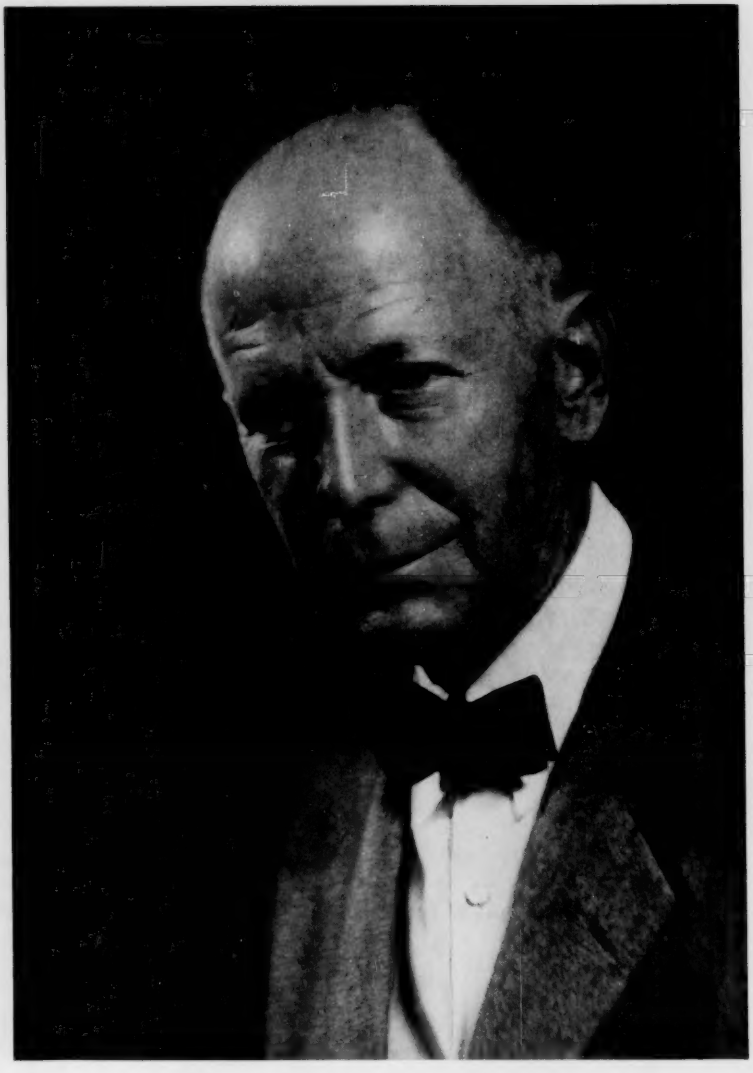

JOHN PUNNETT PETERS 1887-1955

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PAUL H. LAVIETES*

Department of Internal Medicine, Yale University School of Medicine

JOHN PUNNETT PETERS: AN APPRECIATION

Dr. John Punnett Peters, the eldest son of the Reverend John P. Peters and Gabriella Brooke Peters, was born in Philadelphia on December 4, 1887. His mother was an accomplished musician and from her he acquired an enduring love of music. His father, a distinguished biblical scholar and archeologist, received an honorary D.D. from his alma mater, Yale, in 1895 and in the same year was honored by the University of Pennsylvania with an Sc.D. in recognition of his pioneering archeological explorations in the Holy Land. He was active in civic affairs and had a reputation for ruffling the complacency of the members of his wealthy parish through his sermons. His scholarship, broad interests, and sense of social responsibility exerted a profound influence on his son.

John Peters spent most of his preschool years abroad, learning to speak German before English. He attended Trinity School in New York City and then went to St. Johns School, Manlius, New York, from which he was graduated as "top boy" with distinction in English and the classics. He received his B.A. at Yale in 1908 without special honors, although he did achieve a dissertation appointment in his junior year. He acquired some prowess as a gymnast; in his third and fourth years he successfully applied this on the diving board to win a place on the swimming team, which he managed in his senior year. For a year after his graduation he taught English and Latin at St. Johns in order to obtain the means to attend medical school. He also earned some money as a diver for the New York Athletic Club and as an illustrator for a textbook of surgical ophthalmology. The College of Physicians and Surgeons, where he was a member of the honor society, awarded him his medical degree in 1913. He interned at the Presbyterian Hospital in New York for two years thereafter and held the position of Coolidge Research Fellow in Clinical Medicine from 1915 to 1917 and of Instructor in Clinical Medicine from 1916 to 1917 at Columbia.

On May 11, 1917 Dr. Peters was commissioned a captain in the U. S. Army, he embarked for Europe on May 14th, and was at work in Base Hospital Number 2 at Etretat, France, on May 23d. He remained there as officer in charge of the medical service until January 14, 1919, assisting also

* Associate Clinical Professor of Medicine.

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with the anesthesia when casualties arrived in large numbers. He published studies on urinary tract purpura and on prevention of trench foot and worked with a British team investigating cardiac neuroses. While he occasionally spoke nostalgically of some of his experiences during these years and of France, he came away with an intense and lasting feeling about the degrading nature of war.

After his discharge, opportunities for academic work were few and he was preparing for private practice in New York City when he was granted a Research Fellowship in the Russell Sage Institute of Pathology and Instructorship at Cornell for 1919-20. In the following year he had an appointment as Associate Professor of Internal Medicine at Vanderbilt University while doing research at the Rockefeller Institute in New York. In 1921 he became Associate Professor of Internal Medicine at Yale and in 1927 was promoted to the John Slade Ely Professorship and awarded an honorary M.A. He was never chairman of the department, but had a very felicitous relationship with the chairman, the late Francis G. Blake, and served as acting chairman for a year after Dr. Blake retired.

Dr. Peters' investigations before World War I were in the field of respiratory physiology and cardiac dyspnea. He continued these studies after the war at the Russell Sage and Rockefeller Institutes and subsequently at Yale, where they led to an extended investigation of electrolyte and water metabolism. Although his clinical interests were wide, his major concerns were nutritional and endocrine disturbances, renal disease, and toxemias of pregnancy.

Since he was endowed with a keen intellect and remarkable industry, John Peters' accomplishments were inevitable. Actively engaged in clinical research along biochemical lines at the time when analytical techniques applicable to these problems were first developed by Benedict, Folin, and Van Slyke, it seemed natural that he join with his respected mentor, Dr. Van Slyke, in writing a handbook on the subject. Before its completion in 1931 the anticipated handbook had grown into a large two-volume work, *Quantitative clinical chemistry*, which had a tremendous impact and is still the standard work in the field. Dr. Peters read rapidly and critically, abstracted meticulously, and had a prodigious memory. He constantly wrote and revised reviews in the many fields of his interest, primarily to synthesize and clarify his own viewpoints, but many ultimately found their way into textbooks, lectures, and teaching articles for The Yale Journal of Biology and Medicine. His reviews were no mere enumeration and summary of publications but had a flavor and point of view which were extremely stimulating. Incidentally, all of his writing was done in a meticulous script which rarely required revision after typing. He had a remarkable and enviable ability

to pick up his pen whenever there was a free moment, write a few lines or paragraphs, and imperturbably set it down when the house staff came to escort him on ward rounds or when some other duty called.

Dr. Peters was interested in medical care in its broadest sense. To him the special province of the clinical investigator was the study of disturbances created by nature in man, usually more difficult to control and interpret than animal experimentation, but yielding information obtainable in no other way. His investigative work and his teaching were never far removed from the patient. The uncompromising integrity which characterized all of his actions required that scientific principles be applied rigorously to clinical practice. Every patient must have the best medical care available. There was no need for separate wards for investigating metabolic problems. Laboratory services were to be used with discrimination, and only the best techniques available were to be used for all patients. Quantitative data could be collected on all patients in the open wards, thus offering stimulation to students, nurses, and house staff. Surely these were the dreams of an idealist, but Dr. Peters had the realism and tenacity to bring them to fruition. On ward rounds Dr. Peters never confined his attention merely to interesting problems. He took responsibility for every patient on his service and uncovered matters of importance in patients which in some circumstances might be neglected because their problems were not obviously exciting. He was an able clinician, skilled in the over-all care of the patient, and extremely stimulating in his physiologically oriented analysis of their problems. While he was somewhat contemptuous of the so-called art of medicine as a cover for ignorance, he himself was an unusually warm and understanding physician who earned his patients' devotion.

As a teacher Dr. Peters firmly believed that medical students were adults who were not to be spoon-fed but rather stimulated to think. He aimed at the highest level, with the feeling that the less capable would be none the worse and very likely the better for his approach. He met three times with each group of students entering the clinical clerkship in medicine for an informal orientation into disorders of metabolism. Thereafter he met the clerks in even smaller groups for discussions relating to their patients. These were a rewarding experience for the abler students, though sometimes harrowing to the laggards. Dr. Peters disdained didactic lecturing. When he gave lectures to the preclinical classes, they were written with great care and made available to the students, either through Dr. Fulton's library, of which Mrs. Peters was curator, or through publication in The Yale Journal of Biology and Medicine. He saw no reason to repeat the lecture the following year unless rapid advances had occurred. He felt that the Yale Journal should be used freely for articles of teaching value, and he contributed nu-

merous exemplary ones. For most of his time at Yale, Dr. Peters served as faculty adviser to Alpha Omega Alpha and regularly entertained the society and guests at his home after the annual lecture.

Since Dr. Peters was keenly aware that improvement of medical care necessitated that practising physicians have opportunity to continue learning, he engaged in considerable postgraduate teaching. Here, as in his teaching of students, he aimed at stimulating discussion and critical observation and reading rather than formal lecturing. He was especially solicitous of the welfare of men returning to practice after military service. These men spent a profitable day each week with Dr. Peters, making rounds with him in the morning, participating in a luncheon discussion, and seeing clinic patients in the afternoon. Here indeed was postgraduate teaching at its best.

In the matter of provision of medical care, Dr. Peters demonstrated a broad and deep social philosophy. Shortly after the inception of a study of medical care by the American Foundation in the mid-thirties, he was invited to serve on a medical advisory committee which was comprised of individuals who showed particular interest in the study. The result of the project, *American medicine: expert testimony out of court,* indicated recognition by many physicians of a need for change in the organization of medical services. In 1937 a credo, "Principles and Proposals for Improvement of Medical Care," was formulated and a committee to promote it was founded. Organized medicine attacked the Proposals and the committee and successfully blocked any action. The committee continued, however, as a clearing house for information concerning medical care and a source of unfettered expert medical opinion. Dr. Peters was its secretary from 1937 until 1954. His speeches and writings on medical care and his testimony before Congressional committees were based on broad factual knowledge. He was firmly convinced that comprehensive health coverage was necessary and inevitable, and that it was the responsibility of physicians to encourage experimentation in that direction. His great fear was that the medical profession, by blind opposition to change, would forfeit its opportunity to develop the formula to give to the public greater enjoyment of health and to physicians larger opportunities to pursue their chosen calling.

During World War II, Dr. Peters conducted investigations for the Air Force on survival rations and for the National Research Council on nutrition in injury and disease. He was also a Consultant to the Quartermaster Corps. After the war he was appointed Consultant to the Army Medical School and participated in the basic science course as lecturer for four years. In 1947 he was appointed to the Study Section on Endocrinology and Metabolism of the National Institute of Health in the Public Health Service under the Federal Security Agency.

Often outspoken in his defense of unpopular causes, Dr. Peters became the target of reactionary forces in his late years. His fitness to hold his position with the Public Health Service was challenged in 1949 and again in December 1951 on the basis of questionable loyalty. A written answer to the charges served to clear him in 1949 and he was cleared again of essentially the same charges after a hearing in March 1952. In 1953 the Loyalty Review Board of the U. S. Civil Service Commission advised him that they declined to accept the decision of the previous Agency Board of the Federal Security Agency. After a perfunctory hearing on May 12, 1953, he was judged of insufficient loyalty and his appointment with the Public Health Service was terminated.

Hoping to set a precedent that would make it mandatory for anyone accused of disloyalty to be confronted by his accusers in an open judicial hearing, Dr. Peters, at great physical and emotional cost, challenged the validity of this judgment until his case finally came before the purview of the U. S. Supreme Court. To his disappointment, the Court ruled in his favor, not on the constitutional ground of denial of the right to face his accusers, but on the technical ground that the Review Board was not empowered to initiate a re-hearing. The wide editorial acclaim which the victory received throughout the nation was gratifying as an indication that the case had served to point up the serious plight of our Bill of Rights. Throughout the hearings Dr. Peters refused to ask his younger colleagues and friends to serve as witnesses in the fear that they might find their own research grants or positions prejudiced. With characteristic modesty, he minimized his importance in the Court action and spoke of himself only as a bone of contention.

John Peters had a sense of humor and proportion which served him well through many frustrating experiences. He possessed extraordinary patience, bred of faith that firm adherence to principle would ultimately lead to progress, and he had a warmth and humanity which endeared him to those who were close to him and which made it impossible for him to be passive when people were in need.

It could hardly be said of him that he was an extrovert, yet behind his reserve there lay a great joy of life. Art, literature, music, the theater, sports, gardening, good food and wines, and particularly convivial companionship and stimulating discussion all gave him great pleasure. He was a gracious host. He played as enthusiastically as he worked, whether at bridge or the piano, tennis or dancing. Not popular in the trivial sense of the word, John Peters nevertheless had many warm and loyal friends.

Mrs. Peters contributed in no small way to her husband's accomplishments. She took a personal interest in members of his department, helped

with bibliography and proof-reading, readily accepted the curtailing of social life necessitated by the demands of Dr. Peters' work, and was a constant source of encouragement and support. When the children grew to school age, she became librarian of the Laboratory of Physiology where she doubled as editor of the many papers emanating from that department. Here she also helped many students learn to consult the literature and to write clearly. With all of this she was able to maintain an atmosphere of relaxation in the home and enjoyed as much as Dr. Peters entertaining students and colleagues, many of whom became her devoted friends.

John Peters' last years were plagued with illness and anxiety which he bore with quiet dignity. His greatest fear concerning retirement was that he would be deprived of the stimulating associations with younger people, without which he felt he could not work productively. While making ward rounds on October 27, 1955, he had a myocardial infarct and as a result of this died on December 29, 1955 in Wallingford. He remained keen of mind to the end, never losing his courage and kindness, working on his journals and writing and planning for an active future, including private practice and a grant for secretarial aid for continued writing.

He is survived by his wife, Charlotte Hodge Peters, a daughter, Alice Hodge Peters Irwin, three sons, John Hodge Peters, Richard Morse Peters, and Charles Hodge Peters, and eleven grandchildren.

He belonged to the following professional societies: American Medical Association, Connecticut State Medical Society, New Haven Medical Society, Harvey Society, American Association of Biological Chemists, Association of American Physicians, American Society for Clinical Investigation, Society for Experimental Biology and Medicine, American Association for the Advancement of Science, Federation of American Scientists, Interurban Clinical Club. The honorary societies to which he belonged included the National Academy of Sciences, Royal Society of Medicine (London), Nu Sigma Nu, Alpha Omega Alpha, Sigma Xi, Century Club (New York). He served on the Editorial Board of the Journal of Clinical Investigation for many years, and on the Editorial Board of Metabolism from its inception.

The loss of so great a man as Dr. Peters is a poignant one. Few can aspire to his level of accomplishment, but we may all profit by the example of his integrity, faith, and courage. It seems proper to print here a tribute to Dr. Peters delivered at a memorial service on January 3, 1956 by Fowler V. Harper, Professor of Law.

There were two men named Peters—Peters the Scientist, Peters the Citizen. It is not easy to talk about Citizen Peters. He was, in a sense, a bundle of contradictions. He was a gentle and benign man. Yet he could be

hard and stern. And if he spoke softly, it was not that he had little to say nor that he doubted his premises. And although he was a skeptic, he was not a cynic and he was unswerving in his consecration to his principles. Certainly he was a dedicated man—dedicated to science and to the truth as he saw it, and he saw it more clearly than most men.

Dr. Peters was warm in his affection. He gave freely—to his family, to his friends, his students and his patients. But he was also a severe man, stubborn, at times cantankerous. On the other hand, he was tolerant—tolerant even of wicked men, although not of the evil in them. And if his tolerance sometimes failed, it was only at the intolerance and bigotry of others. Nor could he stand hypocrisy or sham in any form. It was William Lloyd Garrison who warned at the masthead of the first issue of The Liberator: "I will be as harsh as truth and as uncompromising as justice." Dr. Peters was that harsh and that uncompromising.

Although by his scientific works Dr. Peters wrote himself into the slender volume of the immortals, he easily attained a humility that lesser men seldom acquire.

I knew Dr. Peters only in the later years but the relationship was one of the most rewarding experiences of my life. I think I saw with some clarity the many contradictions of his character—the kindliness and the steel, the simplicity and the urbanity, the intricate and the complicated personality, the precision-like mechanism of his mind, the softness and warmth, the flint-like quality of his courage. In man's long struggle to civilize himself, it would be hard to find better evidence of success.

My affection for Dr. Peters was deep and abiding for many reasons. But I think it was his fearlessness that made me admire him most. In a world of frightened people, it was a refreshing and invigorating phenomenon. Of course, he came by his courage honestly and naturally. I recall, while preparing for his loyalty hearings, running across a genealogical volume on the Peters family, which has been in this country a long time. In thumbing through it, I discovered a letter written by one Abigail Peters to the presiding judge of the Salem assize, protesting the witchcraft trials. It was impossible to resist the temptation to introduce the letter into the formal record of the loyalty proceedings with the comment that the Peters family has been objecting to witch-hunts for three hundred years. The chairman of the Loyalty Review Board did not appreciate the relevance of the remark as much as Dr. Peters did.

I have heard it said by persons who should have known better that in his nonscientific and particularly his political activities, Dr. Peters was naïve. This was a serious error. It is true that he did not bother to distinguish between good causes and lost ones, but he never mistook a figure of speech for a fact, and although he had many and high ideals, he had few illusions. To call him naïve was to confuse naïveté with courage—a mistake frequently made in these timid days.

Dr. Peters was an individualist. He did not mind if he was in the minority, as he frequently was. He did not mind if he was a minority of one. And yet, he felt strongly about the collective welfare and had definite ideas as to what should be done to promote it. Although his own patients re-

ceived the best medical attention, he knew the American people as a whole did not have adequate medical care and he did everything in his power to improve it. While he reacted violently to the injustices done to a Harry Bridges or an Owen Lattimore, he, at great sacrifice, sought with high determination to improve our political and legal mechanisms to save others from similar devastating experiences.

To be sure, Dr. Peters was neither an infallible scientist nor was he the quintessence of all virtue. He was altogether human. But there are few men who have done more good things for more people with more modesty. With all his serenity of spirit, the sufferings of mankind, individually and collectively, troubled him deeply. We can, I think, appropriately say of him what Tom Paine said of himself—and I know Jack would not object to my quoting Tom Paine on this occasion:

> The world is my country,
> All mankind are my brethren,
> To do good is my religion. . . .

Jack Peters loved life and had a full one. But he faced death as unafraid as he lived. For him, I am sure, it had no sting at all. And with this thought, those of us who loved him should stop grieving at our loss and rejoice in the lasting memory of his friendship.

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WHOLE BODY BUFFERS IN THE REGULATION OF ACID-BASE EQUILIBRIUM

Twenty-five years ago John P. Peters with Donald D. Van Slyke wrote one of the most precise, lucid, and comprehensive accounts of acid-base equilibrium that has ever appeared in scientific literature, namely, Chapter 18 in Volume I, *Interpretations,* of *Quantitative clinical chemistry.*[44] This account rested on the basic experimental work of Van Slyke and other investigators and especially on the classic work of Lawrence J. Henderson[30, 31] who in 1908 first delineated in a unified manner the physicochemical and physiological mechanisms for the maintenance of neutrality of the fluids of the body. In the quarter of a century since the appearance of *Quantitative clinical chemistry,* although much research activity has taken place in this field, no major revision has had to be made of the fundamental concepts as set forth in that work. Nevertheless, the investigative efforts of this subsequent period have greatly enhanced our understanding of both the physicochemical and the physiological mechanisms involved. This is true especially as regards (i) the linked transfers of ions which participate in the functioning of buffer systems, (ii) the rôle of the kidney in acid-base regulation, and (iii) the relationship of disease processes to disturbances of acid-base equilibrium. The first of these three categories is the subject of this paper; the other two are treated in part in some of the following contributions.

The first defense of body fluid neutrality is the physicochemical mechanism of the buffer systems. The buffer systems of the body consist of weak acids and their salts which react with strongly dissociated acids to form a neutral salt and a slightly dissociated weak acid, thus minimizing changes in hydrogen ion concentration. These systems are principally bicarbonates, phosphates, and proteins, and function according to circumstances as either donors or acceptors of hydrogen ions or protons. Once these buffer systems had been identified by Henderson, the attention of investigators was focussed primarily on the buffering activity of the blood.[22, 58, 21, 55] This is easily understandable since blood is such a readily accessible portion of the

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body fluids. On the other hand, studies of buffers in other phases or tissues have not been entirely wanting. Earlier investigators of this aspect of the problem were concerned: (i) with the amount of exogenous acid or alkali which could be tolerated or buffered by the living body or the amount of "nonmetabolic" carbon dioxide* which could be extracted or retained, and (ii) with the extent to which other tissues shared this buffering activity with the blood. More recently, with a greatly expanded knowledge of ionic transfers between multiple phases of the body fluids, investigators have become interested in the quantitative aspects of these ionic exchanges as they relate to whole body buffering of acid-base disturbances.

EARLIER STUDIES OF TISSUE BUFFERING

Henderson[80] clearly stated in 1908 the concept of multiple buffer systems in heterogeneous phases of the body fluids:

> Thus it appears that, through the nature of carbonic acid, and the fact that there is in the body a nearly constant supply of it, this substance possesses a quite wonderful capacity, more than three times as great as any other substance not thus regulated by a constant supply could have, of preserving the reaction in the neighborhood of a hydrogen ion concentration 0.3×10^{-7}.
>
> . . . When, finally, in the process of neutralizing acid, the bicarbonates have been nearly used up, and the reaction is almost precisely at the neutral point, it is evident that protoplasm still possesses in the phosphates substances which have, thanks to the ionization constant of the ion H_2PO_4 (2×10^{-7}), nearly the greatest efficiency which ordinary substances could possess in checking increase of acidity. Moreover the diffusibility† of acid phosphates probably adds to their efficiency, much as the adjustment of carbonic acid tension does to that of the bicarbonates.
>
> . . . The above considerations show that in the matter of neutrality regulation the single phase equilibrium in the body is rendered far more efficient than it could otherwise be by the interventions of other phases acting selectively as reservoirs of supply and as vehicles of escape. Thus the theoretical considerations above developed regarding neutrality regulation must be modified for a heterogeneous system, in so far as relative efficiency of different substances is concerned; for, by the intervention of other phases, substances not possessing the highest efficiency in this process in a single phase system, may become enormously more efficient than any closed single phase system easily surpassed by the heterogeneous system of the body.

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* Although all, or essentially all, carbon dioxide in the body is metabolic in origin, "nonmetabolic" carbon dioxide refers to that portion which is present in the several phases of the body fluids, chiefly in the form of bicarbonate, and which is in dynamic equilibrium with the stream of "metabolic" carbon dioxide steadily flowing from its cellular sources to the organs of excretion. Transfers of hydrogen or of bicarbonate ions, as discussed in this paper, refer, therefore, to shifts in this dynamic equilibrium and not to alteration in a static ionic pattern.

† Henderson includes in this term the renal excretion of monobasic phosphate.

And thus Lawrence Henderson clearly formulated a concept of acid-base regulation which was to be so well supported by the experimental work of the next fifty years.

Investigators of the subject of whole body buffering, following Henderson's formulation, were concerned first with the effects of the *addition of strong acids or alkalies* to the organism (Table 1). Van Slyke and Cullen[41] injected dilute sulphuric acid into dogs by the intravenous route and calculated that five-sixths of the acid was neutralized by buffers outside the blood. Palmer and Van Slyke[43] administered sodium bicarbonate by mouth to human subjects and estimated that its volume of distribution was equivalent, on the average, to 70 per cent of the body weight, i.e., the alkali was buffered in part by systems outside the blood and interstitial fluid. Haldane[28] studied the distribution of ingested ammonium chloride, a "metabolic" acid, by observing its effect on the content of carbon dioxide in blood; he concluded that only about one-eighth of the retained "acid" could be accounted for in an assumed blood volume of five liters. The same problem was approached in a different experimental manner by Banus and Katz;[4] these workers perfused the hind leg of a dog with blood strongly acidified with dilute hydrochloric acid. Since the perfusion resulted in a rise in CO_2 combining power of the blood from the initially depressed levels, without a change in chloride concentration, it was concluded that tissues in the hind leg buffered the acidified blood by at least one of three mechanisms: (i) shift into tissues of an anion other than chloride, (ii) shift out of tissues of cation, or (iii) increase in the protein buffers of the blood. Thus, by 1927 Henderson's concept of heterogeneous phase buffering received ample experimental confirmation in respect to so-called "metabolic" disturbances of acid-base equilibrium.

Investigation of the capacity of tissues and the whole body *to retain or to surrender nonmetabolic carbon dioxide or carbonic acid* when the alveolar CO_2 pressure was experimentally raised or lowered, extended the confirmation of Henderson's concept to "respiratory" disturbances of acid-base equilibrium (Table 2). In 1926 Shaw[60] subjected cats to inhalation of 7.5 per cent CO_2 and found that 84 to 92 per cent of the nonmetabolic CO_2 absorbed was retained in mixed tissues outside a blood volume assumed to equal 5.5 per cent of the body weight. Brocklehurst and Yandell Henderson[10] studied the effects of CO_2 inhalation and hyperventilation in human subjects and calculated that approximately one-half of the capacity of the body to retain or to give up CO_2 lay in the blood. The short duration of their experiment (two to three minutes) may explain the discrepancy between their findings and those of other workers. Adolf, Nance, and Shiling[1] repeated the experiment of CO_2 inhalation for periods of 30 minutes and

TABLE 1. DISTRIBUTION OF WHOLE BODY BUFFERING OF ACUTE EXPERIMENTAL METABOLIC DISTURBANCES OF ACID-BASE EQUILIBRIUM

Investigators	*Experimental procedure*		*No. Expts.*	*Species*	*Av. distribution of buffering*			*Assoc. "intracellular" ionic transfers*—av.*			
					Intravascular (red cell + plasma)	*Extravascular*					*Extracellular reference space*
						Interstitial	*"Intracellular"*	*Na*	*K*	*Anion*	
					per cent of total body buffering			equivalent per cent of total body buffering			
A. Metabolic Alkalosis											
Palmer and Van Slyke[48]	$NaHCO_3$	p.o.	9	Man	13‡		87				
Axelrod, Seip, and Pitts[3]	"	i.v.	6	Dog			43	+18	0	—25 (Cl)	Inulin
van Goidsenhoven, Gray, Price, and Sanderson[60]	"	p.o.	3	Man				+13		—37 (Cl)	Inulin
Singer, Clark, Barker, Crosley, and Elkinton[54]	"	i.v.	7	Man	26	44	30	+35	—15		Chloride
B. Metabolic Acidosis											
Van Slyke and Cullen[61]	H_2SO_4	i.v.	1	Dog	17‡		83				
Haldane[29]	NH_4Cl	p.o.	6	Man	13‡		87				
Schwartz, Jensen, and Relman[46]	NH_4Cl	p.o.†	3	Man	16	29	55	—22	—34		Chloride
Swan and Pitts[58]	HCl	i.v.	5	Dog	18	25	51	—36	—15		Radiosulfate
Tobin[59]	HCl	i.v.	7	Cat				—35	—5	+20 (Cl)	Inulin
								—58	—6		Chloride

The data in this table and in Table 2 have been recalculated in part for purposes of comparison.

* Exclusive of the red-cell phase.

† Subjects depleted of sodium prior to experiment.

‡ See footnote † in Table 2.

calculated that 82 to 97 per cent of the CO_2 "capacity" lay in the tissues outside of the blood. Irving, Ferguson, and Flewes[54] analyzed the CO_2 content of muscle and bone, as well as of blood, from cats subjected to high and low respiratory levels of CO_2; no change in the fairly high CO_2 content of bone was observed and muscle plus blood accounted for only 10 per cent of the nonmetabolic CO_2 lost and only 20 per cent of that retained. Rosenbaum,[46] working in John P. Peters' laboratory, examined the volume of distribution of nonmetabolic CO_2 in human subjects as compared with that of an exogenous "extracellular" tracer substance, thiocyanate. Assuming an unchanged basal respiratory quotient, he calculated that during hyperventilation 21 to 47 per cent of expired nonmetabolic CO_2 came from outside the thiocyanate space, and, during CO_2 inhalation, 12 to 56 per cent of the CO_2 was retained outside this space. More recently Farhi and Rahn[20] have found in such experimental circumstances that two-thirds of the nonmetabolic CO_2 is gained or lost from a phase or phases outside the blood. Thus, it was clearly established that respiratory acid-base disturbances, consisting of deficits or excesses of carbonic acid, are buffered in body fluid phases other than the blood and the freely diffusible interstitial fluid, although the exact tissue sites of these "intracellular" buffer systems were not definitely identified.

RECENT STUDIES OF LINKED IONIC TRANSFERS IN WHOLE BODY BUFFERING

In the studies enumerated above, relatively little attention was paid to the obligatory exchanges of ions associated with the transfers of hydrogen. During the past 15 years the development of the techniques of flame photometry, body phase measurement by dilution of tracer substances, and tissue electrolyte analysis, has led to exploration of this complex subject. In addition, the observations of Darrow and his associates[19, 15] of the association of an extracellular metabolic alkalosis with experimentally produced deficiency of potassium, has focussed attention on the relationship of transfers of this predominantly intracellular cation to alterations in acid-base equilibrium. As a result much new light has been shed on ionic transfers associated with the functioning of the buffer systems of the body.

Acute respiratory disturbances of acid-base balance offer perhaps the best experimental preparation for the study of ionic transfers during buffering since no exogenous load of cations or anions is imposed. Such a study of acute respiratory alkalosis and acidosis in man has been carried out by the author and his colleagues[24] and may be used to illustrate some of the relationships involved. A brief account of these experiments will be pre-

TABLE 2. DISTRIBUTION OF WHOLE BODY BUFFERING OF ACUTE EXPERIMENTAL RESPIRATORY DISTURBANCES OF ACID-BASE EQUILIBRIUM

Investigators	*Experimental procedure*	*No. Expts.*	*Species*	*Av. distribution of buffering*		*Assoc. "intracellular" ionic tranfers*—av.*			*Extracellular reference space*
				Intravascular (red cell + plasma)	*Extravascular (interstit. + "intracell.")*	*Na*	*K*	*Anion*	
				per cent of total body buffering		equivalent per cent of total body buffering			
A. Respiratory Alkalosis:									
Brocklehurst and Y. Henderson[16]	Hyperventilation	5	Man	49†	51				
Irving, Ferguson, and Plewes[34]	"	4	Cat	3†	97				
Farhi and Rahn[35]	"	13	Dog	42†	58				
Giebisch, Berger, and Pitts[38]	"	5	Dog	38	62	+16	+4	—35 lactate)	Radiosulfate
Elkinton, Singer, Barker, and Clark[24]	"	6	Man	30	70	+37	—3	—36 (undeterm.)	Chloride
B. Respiratory Acidosis:									
Shaw[52]	CO_2 inhalation	6	Cat	12†	88				
Brocklehurst and Y. Henderson[10]	"	11	Man	54†	46				
Adolf, Nance, and Shiling[1]	"	6	Man	16†	84				
Irving, Ferguson, and Plewes[34]	"	4	Cat	2†	98				
Farhi and Rahn[35]	"	10	Dog	36†	64				
Giebisch, Berger, and Pitts[38]	"	4	Dog	34	66	—37	—14	+6 (lactate)	Radiosulfate
Elkinton, Singer, Barker, and Clark[24]	"	5	Man	35	65	—47	—9	+9 (undeterm.)	Chloride

* Exclusive of the red-cell phase.
† Underestimated since the "nonbicarbonate" buffering of hemoglobin and plasma protein is not included.

sented here, followed by a summary of similar experiments by other investigators.

One group of six normal human subjects hyperventilated, and a similar group inhaled 7.5 per cent carbon dioxide, for periods of approximately 30 minutes. The effects of this experimentally produced deficit or excess of carbonic acid on the ionic composition of several phases of the body fluids were assessed by assuming normal initial volumes for the phases of red cells, plasma, and interstitial fluid, equating the extracellular fluid with the chloride space, and deriving the internal exchanges of chloride, bicarbonate, hydrogen, sodium, and potassium, from changes in plasma concentration and from external balances (urinary excretion) of these constituents. The central principle of this quantitative analysis of buffer activity is that exchanges of hydrogen ion can be strictly accounted for from changes in extracellular and red cell bicarbonate which occur as the result of loss or retention of CO_2 through the respiratory tract.* This follows from the relationship:

$$HCO_3^- + H^+ \rightleftharpoons H_2CO_3 \rightleftharpoons CO_2 + H_2O$$

Red cell bicarbonate is included with the bicarbonate of plasma and interstitial fluid because of the red cell chloride shift. Thus, if one can measure or calculate the change in total amount of extracellular plus red cell bicarbonate (ΔHCO_{3er}^-) that takes place under a given set of circumstances, and correct this value for the amount of hydrogen retained (primarily bicarbonate lost) by the kidney (b_{H^+})† and for the amount of hydrogen given up or taken up by the reciprocal change in protein nonbicarbonate buffers of the blood (ΔBuf_b^-),† then the balance must be equivalent, ion for ion, with the amount of hydrogen that must have been surrendered or retained by buffer systems in a phase or phases beyond the extracellular fluid (ΔH_i^+):

$$\Delta H_i^+ = \Delta HCO_{3er}^- + \Delta Buf_b^- + b_{H^+}$$

During 30 minutes of hyperventilation the average change in extracellular-red cell bicarbonate (ΔHCO_{3er}^-) in our subjects was —136 mEq. Since only 5 mEq. could be accounted for in the urine and 39 mEq. combined with hydrogen from the protein buffers of the blood, 92 mEq. of

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* See footnote * on page 192.

† In the absence of intake, the balance of hydrogen (b_{H^+}) is calculated as minus the total of the urinary excretion rates of ammonium ion plus titratable acid minus bicarbonate, i.e., bicarbonate excreted with "fixed" cations represents hydrogen ion preserved to the body. Change in the non-bicarbonate buffer anions of blood, hemoglobin and plasma protein, can be calculated from the change in arterial pH. See text of original paper[24] for details of calculation.

bicarbonate must have combined with hydrogen from a source outside this phase to form H_2CO_3 for excretion as CO_2 through the lungs (Fig. 1A). Thus, ΔH_i^+ equalled —92 mEq. and represented 92/131sts or 70 per cent of the extra-renal buffering of the respiratory acid-base disturbance imposed. Conversely, during CO_2 inhalation the average change in extracellular-red cell bicarbonate (ΔHCO_{3er}^-) was +32 mEq. Of the corresponding hydrogen ion released from the retained carbonic acid which was

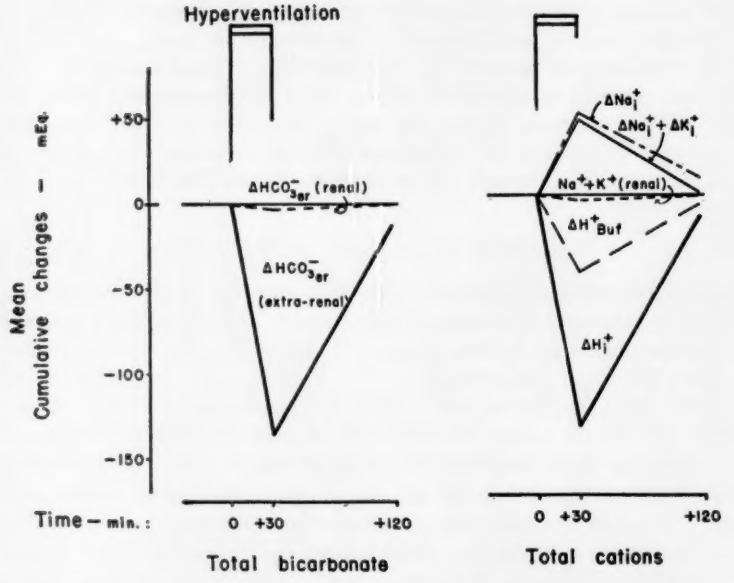

FIG. 1A. Acute respiratory alkalosis: the mean summated patterns of response of total extracellular bicarbonate and of total body cations, to hyperventilation.

Changes are plotted cumulatively from the onset to the end of the stimulus and to the end of the recovery period. On the left the dotted line indicates change in bicarbonate by renal excretion; the solid line indicates the total change in extracellular bicarbonate, the difference being extrarenal or respiratory. On the right are depicted the corresponding changes in cations. The dotted line represents renal excretion of sodium and potassium, the dashed line respiratory loss of hydrogen from blood buffer proteins, and the heavy line total hydrogen loss. The difference between the two latter represents transfer of hydrogen from total body cells. The associated changes in intracellular sodium plus potassium and in intracellular sodium alone are indicated above by the solid and dashed and dotted lines, respectively.

Loss of extracellular bicarbonate by the respiratory route greatly exceeds that by the renal. Associated with the loss of bicarbonate through the lungs is the hydrogen ion drawn in part from the protein buffers of the blood and in part from body cells. The latter decrement is partially replaced by transfer of sodium into cells. (Reproduced from the Journal of Clinical Investigation[24]).

the source of the bicarbonate, 12 mEq. were taken up by blood protein buffers but 2 mEq. were added by renal conservation; therefore, 22 mEq. of hydrogen appeared to have been transferred to buffers in phases other than the extracellular fluid (Fig. 1B). This value for ΔH_i^+ of +22 mEq. represents 22/34^{ths} or 65 per cent of the extra-renal buffering of the acid-base disturbance.

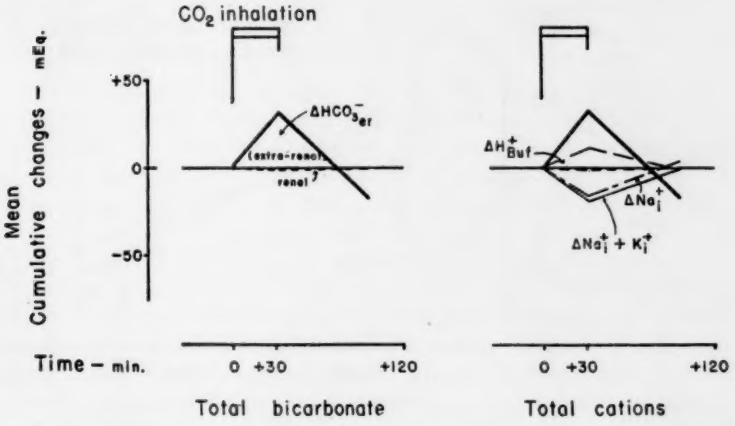

FIG. 1B. Acute respiratory acidosis: the mean summated patterns of response of total extracellular bicarbonate and of total body cations, to CO_2 inhalation. Data are presented as in Figure 1A.

Extracellular bicarbonate is increased in respiratory retention, and hydrogen is taken up by blood protein buffers and by body cells. Simultaneously the body cells lose sodium. (Reproduced from the Journal of Clinical Investigation[84]).

In this type of analysis, transfer of hydrogen ion in one direction cannot be differentiated from an equivalent movement of bicarbonate ion in the other direction; in either case the effect on acid-base equilibrium is the same. What does have to be answered, however, is the question: what are the associated transfers of other ions by which electroneutrality is maintained in the fluid phases involved? Obviously, as in the hyperventilating subject, hydrogen ion cannot enter, or bicarbonate ion leave, the extracellular phase without concomitant decrease in other cations or increase in other anions. Internal exchanges of sodium and potassium were calculated in these experiments by the usual method of multiplying the initial and final volumes of the extracellular phase (as equated with the chloride space) and correcting for the observed external balance of the respective ion. During hyperventilation, when 92 mEq. of hydrogen entered the "extracellular"

fluid from the nonextracellular or "intracellular" phase of the body, 48 mEq. of sodium moved in the opposite direction, potassium transfer was 4 mEq. in the same direction as hydrogen. Conversely, during CO_2 inhalation, when 22 mEq. of hydrogen appeared to have transferred into the "intracellular" phase, 16 mEq. of sodium and 3 mEq. of potassium moved in the opposite direction. The reciprocal relationship of change in "intra-

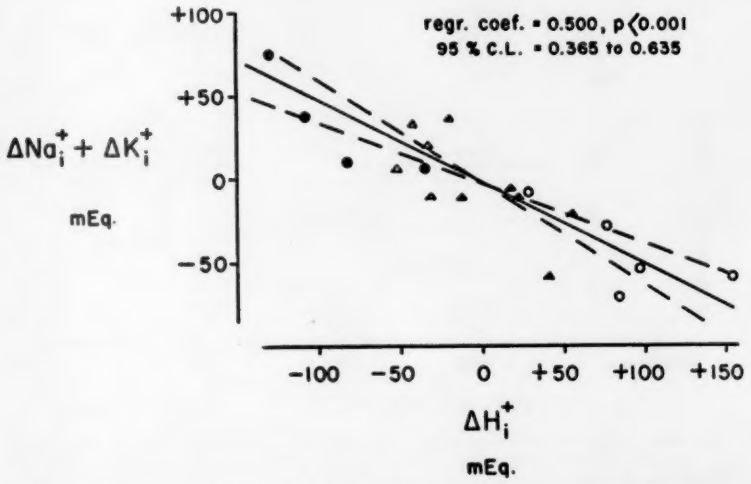

FIG. 2. Acute respiratory alkalosis and acidosis: relation of calculated transfers of cellular hydrogen to those of cellular sodium plus potassium. Solid circles (representing alkalosis) and triangles (representing acidosis) indicate changes during the period of stimulus; open circles and triangles indicate changes from the end of the stimulus to the latter part of the recovery period.

The inverse correlation between the two variables indicates a highly significant reciprocal relationship between transfers of these cellular cations ($r = -0.868$, $p < 0.001$). The relationship, as indicated by the regression coefficient (solid line), is approximately that of two hydrogen ions in exchange for one sodium or potassium ion. (Reproduced from the Journal of Clinical Investigation[24]).

cellular" hydrogen (ΔH_i^+) to the sum of the change in "intracellular" sodium and potassium ($\Delta Na_i + \Delta K_i$) in both experimental and recovery periods of all experiments is shown in Figure 2. The regression coefficient of —0.500 indicates a two for one exchange of hydrogen for sodium or potassium (the exactness of this reciprocal relationship is undoubtedly fortuitous). Since chloride shift was assumed to be restricted to the red cell: extracellular system, the transfer of some other anion with one of the hydrogen ions is the most likely possibility. As discussed below, the experi-

mental data of others indicate that this is probably an organic anion, mainly lactate.

In summary, our experiments are interpreted to show that in acute respiratory alkalosis or acidosis, induced in man for a period of approximately 30 minutes, 94 to 96 per cent of the response was that of extra-renal buffering and only 4 to 6 per cent was due to renal adjustments (the kidney simply had not had time to do more). Of the extra-renal buffering, 65 to 70 per cent was achieved by transfer of hydrogen across the "extracellular": "intracellular" phase boundary, in part in exchange for sodium (predominantly over potassium) and in part with an undetermined anion.

Our findings in humans are quite consistent with the results of the more elegant experiments in dogs of Giebisch, Berger, and Pitts[28] (Table 2). Their study was more definitive in that a greater respiratory acid-base stress was imposed, the renal component of response was eliminated by nephrectomy, and the internal ionic exchanges were calculated in reference to an independent measurement of the "extracellular" phase (as quantitated by the volume of distribution of radiosulfate). Their results may be summarized as follows: in respiratory alkalosis 38 per cent of the buffering could be accounted for by blood proteins (mainly hemoglobin with a chloride shift out of red cells), and 55 per cent by hydrogen transfer into the "readily available" extracellular fluid (35 per cent with lactate and 16 per cent in exchange for sodium plus 4 per cent for potassium transferred out of this extracellular phase). Conversely, in respiratory acidosis, 34 per cent of the buffering was accounted for by blood proteins (with a shift of chloride into red cells), and 57 per cent by transfer of hydrogen out of the "readily available" extracellular fluid (6 per cent with lactate and 37 per cent in exchange for sodium plus 14 per cent for potassium transferred into this extracellular phase). No definitive evidence was found by Giebisch *et al.* for a shift of chloride between the radiosulfate "extracellular phase" and intracellular fluids other than in the red cell. The results of the author and colleagues as enumerated above are surprisingly close to these of Giebisch *et al.*, which clearly define the major internal transfers involved in the whole body buffering of acute respiratory disturbances in acid-base equilibrium.

The exchanges of anions other than chloride between cells and extracellular fluid, under these circumstances, have long been recognized. Small shifts of phosphate were observed in the past[41, 45] and were found in the experiments of Giebisch, Berger, and Pitts cited above. An increase in plasma and extracellular lactate during the respiratory alkalosis of hyperventilation has long been known to be part of the buffering response to this acid-base stress.[5, 41] Recently, the quantitative data of Giebisch *et al.* on this

point have been reinforced by a report from Boucot, Lumb, Mahler, and Stanbury[9] to the effect that in acute respiratory alkalosis any fall in arterial plasma carbon dioxide in excess of 5 mM. per liter was accompanied by a nearly equivalent rise in plasma lactate; pyruvate rose as well and phosphate fell. These findings were interpreted to indicate that a glycolytic process is involved in the buffer activity of tissue cells.

Internal ionic exchanges which occur in the buffering of *acute metabolic disturbances* of acid-base equilibrium have also been the subject of recent experimental study (Table 1, A). When sodium bicarbonate was infused into nephrectomized dogs, Axelrod, Seip, and Pitts[8] observed that some 43 per cent of the alkali was buffered by ion exchange between the "extracellular" phase as measured by the volume of distribution of inulin and the remaining "intracellular" fluid; of this portion some 18 per cent was buffered by transfer of sodium with bicarbonate into the "intracellular" phase and 25 per cent by exchange of "intracellular" chloride for "extracellular" bicarbonate. Similar results were found by van Goidsenhoven, Gray, Price, and Sanderson[60] in a more chronic experiment in human subjects who ingested sodium bicarbonate for a period of days. Again, as calculated in reference to the inulin space, an average of 13 per cent of the retained sodium and bicarbonate transferred beyond this space and 37 per cent of the bicarbonate exchanged for chloride between the two phases. Singer, Clark, Barker, Crosley, and Elkinton[54] calculated the distribution of sodium bicarbonate acutely infused in hypertonic solution in man. At the end of two hours, of the retained bicarbonate (as buffer anion or its cationic equivalent), 26 per cent on the average was distributed in blood, 44 per cent in extravascular "extracellular" fluid, and 30 per cent in "intracellular" fluid. Since the ionic exchanges were quantitated in reference to an "extracellular" phase equated with the chloride space and calculated from the chloride balance, no exchanges of bicarbonate for "intracellular" chloride were derived; nevertheless, some two-thirds of the "intracellular" transfer of bicarbonate was accounted for by a simultanous net movement of cations in the same direction (35 per cent sodium in, minus 15 per cent potassium out). The problem of the identification of the "true" extracellular space in reference to which internal ionic transfers should be calculated is taken up later. However this phase is defined, it is clear that buffer systems in other phases of the body fluids are operative in acute metabolic alkalosis.

There is experimental evidence that such is likewise the case in *acute metabolic acidosis* (Table 1, B). Schwartz, Jensen, and Relman[48] administered ammonium chloride as an acidifying salt to human subjects previously depleted of sodium; 16 per cent of the retained hydrogen ion was

accounted for in the blood, 29 per cent in the extravascular "extracellular" fluid as defined by the chloride space, and 55 per cent in the remaining "intracellular" phase. The latter was associated with a loss from this phase of an equivalent 22 per cent of sodium and 34 per cent of potassium, the predominance of the potassium possibly being due to the sodium-depleted state of the subjects. Schwartz, Ørning, and Porter[49] investigated the proportionality of extracellular to intracellular buffering of hydrochloric acid infused into dogs in varying amounts. They found that, when time was allowed for equilibrium to be reached, the relative degree of buffering in each phase remained constant regardless of the load of acid imposed; no actual values for the buffer activity of the several phases were given. Swan and Pitts[58] studied the neutralization of hydrochloric acid infused into nephrectomized dogs; about 18 per cent of the hydrogen was buffered in the blood, 25 per cent in the remaining "extracellular" fluid as defined by the volume of distribution of radiosulfate, and 51 per cent in the "intracellular" phase by exchange for sodium (36 per cent) and potassium (15 per cent). In these experiments, in which an independent measurement of "extracellular" fluid was made other than with inulin, no definitive shift of chloride was observed other than with the red cell. Tobin[59] performed similar experiments in nephrectomized cats using both inulin and chloride as "extracellular" reference spaces. Using the inulin space, 35 and 5 per cent of the added hydrogen were exchanged for sodium and potassium, respectively, which entered the extracellular phase, and 20 per cent was transferred out of this phase with chloride; in respect to the chloride space, 58 per cent of hydrogen was exchanged for sodium and 6 per cent for potassium from outside this space. In both sets of calculations Tobin estimated that 22 to 24 per cent of the added hydrogen combined with bicarbonate (presumably expired) and some 14 to 16 per cent of the buffering was accounted for. Since this latter must include buffering by blood proteins and since part of the chloride shift undoubtedly took place into the red cell, the distribution between intravascular and extravascular phases of the total body buffering in these experiments is not presented in Table 1, B.

In summary, metabolic disturbances of acid-base equilibrium, like respiratory disturbances, are buffered by systems in multiple phases of the body fluids including one or more phases which lie beyond those ordinarily considered to be extracellular in location. Such buffering involves internal exchanges of sodium and potassium ions for hydrogen ions and, under some circumstances, chloride and/or other anions for bicarbonate. In this way electroneutrality is maintained, while hydrogen ion concentration is defended, in this heterogeneous phase system.

CURRENT PROBLEMS AND THE EVIDENCE FROM TISSUE ANALYSES

Despite the fact that investigations of this complex physicochemical and physiological phenomenon over the past half-century have amply confirmed and elaborated the concepts of Lawrence Henderson, many problems remain to be clarified.

The problem of quantitation of the total acid-base disturbance underlies the interpretation of all the data presented in the foregoing portion of this paper. Variation in method of this quantitation undoubtedly explains some of the discrepancies between the results as shown in Tables 1 and 2. In the study of respiratory disturbances of acid-base equilibrium, two main and different methods have been employed. One method has been to measure directly the total amount of carbon dioxide that has been retained or given up, i.e., the external balance of carbon dioxide, and to correct this value for its simultaneous metabolic production. This method is subject to error because of uncertainty as to this correction, uncertainty which is due both to the relative magnitudes of the two fractions of carbon dioxide and to possible variation in the basal respiratory quotient during the experimental stimulus. The other method has been to measure the change in amount of bicarbonate in the extracellular fluid (interstitial fluid plus plasma) and red cells and to calculate from this change the total buffer activity and the associated internal ionic transfers. This latter method must yield incorrectly low values to the extent of any change occurring in intracellular nonmetabolic bicarbonate; and Rosenbaum's[49] experiments cited above indicate that this may be a very significant moiety. Since the data of Giebisch *et al.*[25] and of the author and his colleagues[24] were derived by this second method, it is probable that the calculated proportion of intracellular buffering is underestimated in these studies.

The problem of identification of the extracellular phase that should be used as a reference point for calculation of transfer of "intracellular" ions is only too well known to every student of the field and does not need to be discussed in detail here. Recent evidence for a connective tissue sub-phase of extracellular fluid into which inulin penetrates much more slowly than does chloride or bicarbonate[40, 16] casts suspicion on ionic transfers, and especially on those of chloride, calculated in reference to the inulin space. On the other hand, the possibility that in at least some circumstances or in some tissues (such as skin) chloride may penetrate cells[20, 27] renders suspect ionic transfers calculated in reference to the chloride space. For these reasons, the interpretation of data concerning internal exchanges of ions must always take into account the particular "extracellular" reference phase employed in the derivation.

The characterization of *the buffering capacity and associated ionic transfers of specific organs or individual tissues* is a likely route to further clarification of this problem although the difficulty of the "extracellular" reference phase is still present. Despite the fact that the emphasis in this paper has been on the buffer response to acid-base stress of the total organism, experimental approach to the problem on the tissue level is not new. In 1928, for example, Fenn[26] compared the CO_2 dissociation curves of nerve and muscle with those of blood at different levels of CO_2 pressure and found the buffering capacity of those tissues to be one-third to one-half that of blood. The buffering of acidified blood by perfused hind limb of the dog by Banus and Katz[4] has already been referred to, as well as have the tissue analyses of muscle and bone under similar circumstances by Irving *et al.*[34] Bone particularly deserves to be investigated because of the recent evidence that at least part of the sodium in this tissue exchanges readily with that in extracellular fluid under a variety of circumstances that have been reviewed by Bergstrom[6] and by Nichols and Nichols.[30] In addition to exchanges of fixed cations of bone, carbon dioxide in the form of carbonate on the crystal surface may be transferable under conditions of acid-base stress. This is suggested by studies such as that of Irving and Chute[33] who found a loss of 6 to 14 per cent of bone carbonate in animals made acidotic with hydrochloric acid, and that of Bergstrom[5] who observed that the bones of acidotic rats lost carbonate and Na + K in equivalent amounts.

Data have been rapidly accumulating on changes which occur in the electrolyte content of skeletal muscle in acid-base disturbances. Of these the least well documented are the changes in intracellular bicarbonate and pH. Wallace and Hastings[42] made such measurements in the muscle of cats infused with strong acid or alkali; they found essentially no change in intracellular bicarbonate at the times when the extracellular concentration of the ion was elevated or depressed and estimated that the intracellular pH was conditioned mainly by changes in CO_2 pressure or H_2CO_3 concentration. On the other hand, Gardner, MacLachlan, and Berman[27] observed a decrease in intracellular bicarbonate, and hence pH, in rats experimentally depleted of potassium, a finding consistent with the hypothesis of intracellular acidosis in this condition (see below). More information is available concerning the distribution in muscle of sodium and potassium, under these conditions. Darrow and associates[18, 19] found an increase in intracellular sodium in rats with metabolic alkalosis and a decrease in metabolic acidosis with reciprocal changes in cellular potassium. Cotlove *et al.*[17] calculated the fixed cation content of the intracellular phase of muscle in rats similarly treated. In the alkalotic rats, cellular potassium decreased and sodium increased but only in amounts equivalent to two-thirds of the

potassium lost, the total content of fixed cation (Na + K + Mg) being reduced; in the acidotic animals sodium decreased and potassium increased to a comparable extent in this phase. Levitt *et al.*[39] found that acidosis in rats mobilized sodium from bone cortex, tendon, and muscle with no significant changes in potassium content of the latter tissue. Tobin[57] in the experiments cited above and presented in Table 2 compared the data for the whole intact animal with those from muscle analyses in respect to shifts of sodium and potassium between the inulin space and "non-inulin" space; the loss of sodium from the "intracellular" phase of muscle accounted for two-thirds of the increase in "extracellular" sodium, the loss of "intracellular" potassium of muscle exceeded that found in the inulin space. Thus it is evident that the experimental data from tissue analyses are reasonably consistent with the data from studies of the intact organism in respect to heterogeneous phase buffering but are not nearly complete enough to define the exact rôle of buffer systems in specific tissues.

At this point it is necessary to comment on *the particular problem of the association of intracellular depletion of potassium with extracellular alkalosis;* does this phenomenon have some relationship to the internal ionic exchanges which are involved in whole body buffering of acid-base disturbances? This association, first described by Darrow and his co-workers,[19] has been amply confirmed in rats and in humans, but the factors which condition it have not been entirely clarified. Darrow's group showed[14] that it was not due simply to reciprocity of transfers of hydrogen and potassium in the renal tubule,[8] but that an internal shift of hydrogen with sodium into the "intracellular" phase of the body must be involved, a finding confirmed by Orloff, Kennedy, and Berliner.[42] Analysis of the electrolyte composition of rat muscle in respiratory acidosis by Cooke, Coughlin, and Segar[18] then indicated that in these circumstances of an elevated extracellular bicarbonate and total CO_2 content but depressed pH, intracellular potassium was if anything increased and intracellular sodium was diminished; cellular cation transfers therefore appeared to be related to changes in extracellular pH rather than to extracellular bicarbonate content. Nevertheless, potassium depletion of a severe degree may exist without a fixed relationship to intracellular sodium retention[47, 12] and without an accompanying extracellular alkalosis.[50, 56] In our laboratory,[56] normal human subjects maintained on a daily intake of potassium of less than 1 mEq. for as long as two to three weeks, and developing a total deficit up to 500 mEq. of the ion, have uniformly failed to show any signs of alkalosis even when high loads of sodium were given and but inconstantly when desoxycorticosterone was administered. Perhaps chloride subtraction as well as sodium load or adrenocortical hyperactivity, as suggested by Moore *et al.*,[38] is a necessary factor.

This problem is discussed in detail in a subsequent contribution in this volume.

In any case, the important point about this phenomenon in respect to whole body buffering, in the author's opinion, lies in the primacy of the stimulation. Given such a system of linked ionic transfers between multiple fluid phases, the precise transfers that take place depend upon the nature of the primary stimulus. If the primary event is the development of an extracellular alkalosis, respiratory or metabolic, hydrogen moves from the "intracellular" phase into the extracellular at least in part in exchange for sodium, and potassium transfers are minimal. Conversely, if the primary event is the development of an extracellular acidosis, the opposite occurs, namely, hydrogen moves into the "intracellular" phase mainly in exchange for sodium. Transfers of intracellular sodium appear to predominate over those of potassium in exchanging for hydrogen under these circumstances, despite experiments indicating that the extracellular *concentration* of potassium may be altered;[35, 51, 11] prior depletion of the organism of sodium[48] or potassium[17] may modify these cationic relationships. On the other hand, when intracellular potassium depletion is the primary event, extracellular hydrogen and sodium may transfer together into cells and so create or exacerbate a concomitant extracellular alkalosis rather than buffer or defend the hydrogen ion concentration of this phase. This concept of the linked ionic transfers which are involved in the body's defense of neutrality in its several fluid phases has been presented diagrammatically elsewhere.[35]

In this discussion little has been said of the rôles of the lungs and kidneys in the physiological regulation of acid-base equilibrium. Obviously, the physicochemical defense of neutrality by the buffer systems of the whole body is in itself a self-limiting process; continuous function of this process depends entirely on modification of these buffer systems by ionic exchanges with the external environment. Such modification by regulatory organs must therefore be the major determinant of acid-base equilibrium and of ionic composition in health and in states of disease, and the rôle of the kidney is discussed in the next paper. Nevertheless, the physicochemical process of whole body buffering is an integral part of the body's response to acid-base stress, and as such is well worth further investigation.

SUMMARY

The development of the concept of whole body buffering in multiple heterogeneous phases of the body fluids has been reviewed. Early studies of this phenomenon have been cited which indicated that a major portion of this physicochemical defense of body fluid neutrality took place in phases other than that of blood. More recent data have shown that such multiple

phase buffering involves a series of linked ionic exchanges. These exchanges include those of hydrogen, sodium, potassium, and certain anions, and result in the maintenance of electroneutrality in the face of redistribution of the hydrogen ion. Many problems remain in respect to quantitation of these processes, to definition of fluid phases, and to precise delineation of these exchanges in particular tissues such as muscle, nerve, and bone.

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THE RÔLE OF THE KIDNEY IN THE REGULATION OF ACID-BASE BALANCE

It would be presumptuous to ascribe a major interest to John Peters. His field was all of human physiology. And yet it would not be remiss to consider his contribution to the understanding of acid-base balance as among his most significant efforts. Today, 25 years later, little can be added to the exposition of CO_2 transport in blood, the nature of buffers, and the reaction to acidosis and alkalosis as described in *Quantitative clinical chemistry.*[37] The rôle of the kidney in the maintenance of the electrolyte equilibrium of the body was only beginning to be understood in the 1930's and much has transpired since the publication of Peters' early papers. His continuing interest in this aspect of investigation can be measured in part by the number of his former associates and students who, stimulated and prodded by his penetrating questions, have concerned themselves with renal physiology.

It will be the purpose of this paper, not to review in detail the mechanism of the renal regulation of acid-base balance—this has been the subject of several excellent papers[19, 28, 29]—but rather to discuss a few of the more recent contributions in an effort to delineate some of the problems, emphasizing those of a controversial nature.

THEORIES OF ACIDIFICATION

The primary function of the kidney in the regulation of acid-base balance is to maintain electrolyte equilibrium. Thus, although acute disturbances of acid-base balance, whether of metabolic or respiratory origin, are automatically countered by buffers of blood and tissues and by variations in the excretion of CO_2 by the lungs, excesses and deficits of fixed cation or anion must in the final analysis be corrected by differential excretion by the kidney.

Urinary acidification is characterized by the excretion of both titratable acid and ammonium salts. In Peters' earlier years it was thought that all of the excreted acid was originally present in the glomerular filtrate and that net acidification was achieved through preferential reabsorption of the

* Senior Investigator.

alkaline moiety of the urinary buffer pairs (Na_2HPO_4/NaH_2PO_4 and $NaHCO_3/H_2CO_3$). The substitution of ammonium chloride for sodium chloride was thought to be an independent process.

Pitts and his associates in a series of classical papers demonstrated the inadequacy of this formulation by showing that neither bicarbonate nor dibasic phosphate reabsorption could account for the high rates of titratable acid excretion noted in metabolic acidosis.[40, 41, 48] They concluded, as Smith had postulated before them,[51] that hydrogen ions must be added to urine in excess of that filtered, and considered this to be accomplished by extrusion of hydrogen ions into tubule urine in exchange for filtered sodium. This process accounts quantitatively for the excretion of titratable acid and of ammonia and for the reabsorption of bicarbonate in the following manner: (a) titration of alkaline buffer salts to their acid form thereby increasing titratable acid excretion (e.g., $Na_2HPO_4 + H^+ \rightarrow NaH_2PO_4$); (b) establishment of a hydrogen ion gradient* between cells and urine with resultant accumulation of ammonium ion; (c) interaction of secreted hydrogen ion with urinary bicarbonate ion, formation of carbonic acid, and its dissipation as CO_2 and water. In all of the above processes sodium and bicarbonate are ultimately transported into the blood. Figure 1, modified from Pitts, summarizes the salient features of this theory. The rôle of the carbonic anhydrase system differs from that postulated by Pitts and will be discussed below.

Despite general acceptance of this view of urinary acidification, it must be recognized that there is no direct evidence for the existence of an ion exchange process in renal tubule cells. Furthermore, at least two other theories are consistent with the experimental observations. Menaker, in Peters' laboratory, pointed out that reabsorption of carbonate ion from glomerular filtrate will account for observed rates of hydrogen ion excretion.[50] However, since considerable uptake of carbonate, an ion present in vanishingly low concentration in urine, would be necessary to effect maximal rates of hydrogen ion excretion, Pitts considers this mechanism improbable.[50] His objection, though valid, is not necessarily critical. More recently Brodsky has postulated that sodium bicarbonate reabsorption in an area permeable to CO_2 is equally compatible with the data.[10] It is his view that sodium bicarbonate may be reabsorbed by an active process. The resultant fall in pH would lead to the interaction of carbonic acid with nonbicarbonate buffers forming titratable acid and more sodium bicarbonate. CO_2 from blood would then diffuse into urine along the new concentration

* The maximal H^+ ion gradient between plasma and urine is approximately 1000:1, that between cells and urine is unknown.

gradient, interact with water to form carbonic acid and thereby serve as a continuing source of hydrogen and bicarbonate ions. Although all degrees of urinary acidification could be achieved by a cyclical process of this nature, the hypothesis has a number of limitations. In the first place it requires that both sodium and bicarbonate ions be reabsorbed by active processes, rather than sodium alone as in Pitts' hypothesis. Though a positive intraluminal electrical potential difference produced by bicarbonate ion abstraction could effect the passive movement of sodium out of urine, this is unlikely in view of available measurements of lumen potential, all of which have been distinctly negative.[59] Furthermore, Berliner has pointed out that the rate-limiting reaction in Brodsky's system is in all probability the hydration of CO_2 in urine.[8] The speed of this reaction is insufficient in the absence of carbonic anhydrase to account for observed rates of acid excretion in metabolic acidosis.* Despite these objections, it must be reiterated, as with the Menaker hypothesis, that none is sufficient in itself to exclude the existence of the postulated mechanism. However, there is good evidence for specific and active reabsorption of sodium in the kidney. The specificity, as Ussing has pointed out, is strongly suggestive of transport by combination with a carrier.[56] Since this is most readily visualized as accomplished by a process of ion exchange, Pitts' hypothesis represents the most reasonable concept of urinary acidification.

One of the findings interpreted as supporting the view that hydrogen ion is secreted into urine is the elevation of the CO_2 tension of alkaline urine above that of plasma. The precise mechanism of this rise has been the subject of considerable debate.[15, 24, 32, 41] Pitts has assumed that the excess CO_2 in urine is derived from carbonic acid formed by the secretion of hydrogen ion into bicarbonate containing tubule fluid (Fig. 1 (c)). Since renal tubule cells are believed to be freely permeable to dissolved CO_2, observed elevations in the CO_2 tension of urine (the sum of dissolved CO_2 + carbonic acid) are ascribed to the delayed dehydration of this carbonic acid in the absence of carbonic anhydrase.[41] Mainzer[27] and Clark,[11] on the other hand, assumed that elevations in urine CO_2 tension above plasma were due to admixture in the bladder of urines formed at different times and of differing pH, the excess CO_2 being produced by titration of the bicarbonate of the less acid urine. Kennedy, Orloff, and Berliner were able to exaggerate

* It may be recalled that the equilibrium ratio of the concentration of dissolved CO_2 to carbonic acid in the body is 800:1. Only carbonic acid interacts with buffers. The speed of attainment of equilibrium in the absence of carbonic anhydrase is slow, requiring 200 seconds to come within 10 per cent of equilibrium at 38° C.[49] Though urine does not contain appreciable amounts of carbonic anhydrase, the possibility that the enzyme is present in the luminal membrane cannot be excluded.

the difference between the CO_2 tensions of urine and plasma by increasing the concentration of buffer in urine.[24] They suggested that urine pH differs in individual tubules and that admixture occurs above the renal pelvis. The surface-volume relationship in this area was thought to be unfavorable for free diffusion of CO_2 into plasma so as to account for the observed gradient.

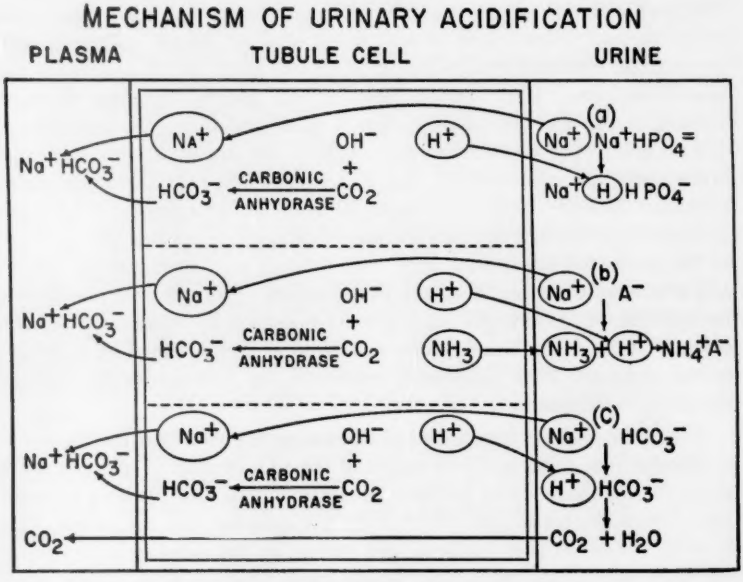

FIG. 1. (a) Formation of titratable acid, (b) formation of NH_4^+, (c) reabsorption of filtered bicarbonate. Carbonic anhydrase is not depicted as involved directly in the transport process; it catalyzes either the interaction between CO_2 and OH^- or the hydration of CO_2. In the latter instance H_2CO_3 can then neutralize OH^-.

Added evidence for the mixing hypothesis was adduced by varying urine flow, in which instance urine CO_2 tension diminished with increasing flow due to the lowering of buffer concentration. This is counter to the delayed dehydration hypothesis since a decrease in urine transit time would further diminish the time available for the dehydration of carbonic acid.

Recently Ochwadt and Pitts demonstrated that an elevated CO_2 tension of urine may be reduced to the level of that of plasma by addition of carbonic anhydrase to urine through its intravenous administration.[88] While indicating that the delayed dehydration of carbonic acid does play a part in the elevation of urine CO_2 tension and, indeed, that the excess carbonic acid

involved is formed in an area permeable to CO_2, these experiments in no way define the mechanism of the formation of the carbonic acid. The elevation of CO_2 tension produced by mixing buffered urines of differing pH also involves carbonic acid as an intermediate in the process. The interpretation that the excess carbonic acid is formed by hydrogen ion secretion leaves unexplained the effect of urine buffer concentration, the effect of urine flow, and the reported observation of urine CO_2 tensions below those of plasma in acid urines of low buffer content. It is more likely that excess carbonic acid is formed by the mixing of urines of disparate pH in a region of tubule permeable to CO_2 (e.g., collecting tubules, collecting ducts). Under such circumstances addition of carbonic anhydrase would accelerate the dehydration of carbonic acid to CO_2 and water and reduce the CO_2 tension to that of plasma.

SOURCE OF URINARY HYDROGEN ION AND THE RÔLE OF THE CARBONIC ACID SYSTEM

It is widely held that hydrogen ion secreted into the urine is derived from intracellular carbonic acid formed by hydration of CO_2.[28, 40, 47] There is no evidence for this hypothesis and it may represent a misleading oversimplification. Data relating changes in plasma CO_2 tension and urinary acidification afford no information as to the source of extruded hydrogen ion.[8, 10, 45] Though intracellular carbonic acid concentration rises when plasma CO_2 tension is elevated, it cannot be determined whether this provides more substrate for the acidification process or exerts an effect by lowering cell pH. Similarly, experiments relating renal CO_2 production and hydrogen ion excretion are not pertinent in defining the origin of urinary acid.[47] CO_2 undoubtedly plays a rôle in the acidification process insofar as acid-base balance is concerned since, as noted in Figure 1, bicarbonate is formed whenever ammonia or titratable acid is excreted or bicarbonate reabsorbed. However, there may be appreciable secretion of hydrogen ions in the absence of acidification.* Thus it has been suggested that the great bulk of filtered sodium is reabsorbed by exchange with hydrogen ion.[2] Since urine pH does not change in the proximal segment, sodium may either exchange with hydrogen and chloride with hydroxyl ion or alternatively chloride be passively reabsorbed as a consequence of a negative electrical potential

* The actual turnover of hydrogen ions in cells and urine is of no consequence insofar as acid-base balance is concerned. Of importance is the net loss of acid from the body, estimated as the sum of the rates of excretion of titratable acid plus ammonia minus that of bicarbonate. The distinction between net acidification and rate of hydrogen ion secretion is of value, however, in evaluating the significance of a number of situations. Changes in acid excretion and hydrogen ion extrusion do not necessarily parallel each other and it is frequently difficult to estimate the relative rates of the latter on the basis of urinary data alone.

induced by active sodium transport followed by back diffusion of hydrogen ion. In either instance, so long as a steady state is maintained, neither luminal nor cell pH will change, nor will bicarbonate ion be produced.

Secreted hydrogen ion may be derived from other metabolic reactions. The rapid extrusion of hydrogen ion from cells would lead to a rise in cell pH and consequent interference with acidification, except in the isohydric segment as noted above, unless hydroxyl were disposed of first by cellular buffers and then by transport across the contraluminal border. The carbonic acid system in effect may serve both purposes, a ready source of disposable buffer minimizing cell pH changes and an indirect mode of hydroxyl transport. Davies and Roughton have commented on the rôle of CO_2 as a buffer with respect to the stomach,[18] and Jacobs and Stewart on that of bicarbonate as an hydroxyl transport system in red cells.[28] In this view, carbonic anhydrase enhances the buffering efficacy of the $CO_2/H_2CO_3/HCO_3^-$ system by catalyzing the formation of bicarbonate from CO_2 and hydroxyl ion; bicarbonate may then be transported into plasma.* This is depicted in Figure 1. Inhibition of carbonic anhydrase will interfere with acidification by diminishing the efficiency of the CO_2 buffer system, thereby allowing the development of intracellular alkalosis. Parenthetically, it may be stated that it matters little what the ultimate source of urinary hydrogen ion is insofar as net acid excretion is concerned. Whether it be derived from the hydration of metabolic CO_2 or from any other hypothetical process, in either instance, the rate limiting step in the acidifying process is the hydration of CO_2. However, the recognition that CO_2 probably serves essentially the same function in tubule cells as in other systems (e.g., red cells) is of importance.

THE SOURCE OF CO_2

Roberts *et al.* noted that the maximal rate of hydrogen ion excretion (defined by them as the sum of titratable acid plus ammonia excretion) in the dog approximated but never exceeded total renal CO_2 production.[47] They interpreted this as indicating that locally produced CO_2 is involved in the acidification process. However, the data actually constitute convincing evidence that CO_2 utilized in the secretory process must originate from other sources as well. Since only a fraction of renal cells is engaged in the formation of titratable acid and ammonia, CO_2 of necessity must be added to these cells from the plasma to account for the observations. Furthermore, their definition of hydrogen ion secretion omits from consideration hydro-

* Whether carbonic anhydrase catalyzes the reaction
$CO_2 + H_2O \rightleftharpoons H_2CO_3$ or
$CO_2 + OH^- \rightleftharpoons HCO_3^-$ is of no consequence since in the first instance the dissociation of H_2CO_3 is instantaneous, making a distinction between the processes impossible.

gen ion expended in converting filtered bicarbonate to carbonic acid. Much more hydrogen ion may be used in this process than in the formation of titratable acid or ammonia. Consequently this would impose on the secretory cells an even greater requirement for metabolic CO_2 production. Urinary carbonic acid derived from the interaction of hydrogen and bicarbonate ions in the reabsorption of bicarbonate cannot contribute CO_2 to the pertinent cells inasmuch as the dehydration of carbonic acid presumably occurs in an area distal to the acidifying segment.

TABLE 1. RELATIONSHIP BETWEEN INTRACELLULAR HYDROGEN AND POTASSIUM CONCENTRATION AND URINARY ACIDIFICATION

	Cell		*Urinary excretion*				*Urine*
	pH	*K+*	*T.A.**	NH_4	*K+*	HCO_3^-	*pH*
Respiratory acidosis	Decr.†	Norm.‡	Incr.§	Incr.	Decr.	Decr.	Decr.
Hypokalemic alkalosis	Decr.	Decr.	Incr.	Incr.	Decr.	Decr.	Decr.
Respiratory alkalosis	Incr.	Norm.	Decr.	Decr.	Incr.	Incr.	Incr.
Potassium infusion	Incr.	Incr.	Decr.	Decr.	Incr.	Incr.	Incr.
Acetazolea-mide admin-istration	Incr.	Norm. or Decr.	Decr.	Decr.	Incr.	Incr.	Incr.

* Titratable acid. † Decrease. ‡ Normal. § Increase.

FACTORS INFLUENCING THE SECRETION OF HYDROGEN AND POTASSIUM IONS

Despite limited information concerning either the source of hydrogen ion or its mode of transport into urine, a number of factors are known which influence its rate of secretion. Furthermore, since potassium is also secreted,[4, 81] presumably in exchange for sodium, it is not surprising that factors which influence acidification may also exert effects on the excretion of potassium. The intracellular concentration of hydrogen ion appears to be an important determinant of the secretory rates of these ions.[2, 5, 85] Factors which can be assumed to decrease cell pH enhance the excretion of titratable acid and ammonia and diminish that of potassium and bicarbonate. The converse is observed when cell pH is presumably elevated. The relationship between cell hydrogen ion content and the respective urinary changes for a number of situations to be discussed below is summarized in Table 1. The intracellular content of hydrogen and potassium in renal tubule cells has not been determined analytically, but assumed on the basis of inferential evidence. It should be noted that the urinary changes are elicited even when cell potassium content is presumably normal. Though the reciprocal

changes in the excretion of potassium and hydrogen ions observed in hyper- and hypokalemia may be conditioned largely by variations in intracellular potassium content, changes in cell pH induced by transcellular shifts of potassium and hydrogen ions may also play an important rôle. In any event, cell pH is thought to be altered in all of the situations listed in Table 1, whereas cell potassium content is presumably unchanged in the respiratory modifications of acid-base balance and during inhibition of carbonic anhydrase activity.

The importance of the filtered load of bicarbonate and nonbicarbonate buffers in the process of acidification cannot be minimized. The composition of glomerular filtrate in this regard is obviously a major factor insofar as net acidification is concerned. It may affect the secretory rates of hydrogen and potassium ions apparently in the absence of changes in the cell content of either ion; whether the filtered load exerts an independent influence on the maximal hydrogen ion gradient that can be established between urine and plasma is unknown. Net transport of hydrogen ion apparently ceases when urine pH approximates 4.5, but the mechanism is unclear.[88] Maximal pH on the other hand need not be produced by secretion of alkali but may be explained by concentration of urine bicarbonate. Thus abstraction of water from bicarbonate containing urine increases the concentration of bicarbonate to a greater extent than that of the diffusible CO_2, consequently pH's above that of plasma may be achieved.

PLASMA CO_2 TENSION AND ACIDIFICATION

The relationship between plasma CO_2 tension and acidification has been commented on before[85] and experimentally defined by a number of workers.[8, 10, 45] Though the changes have been ascribed to alterations in the carbonic acid content of renal tubule cells, it has not been possible to exclude with certainty the influence of variations in total body acid-base balance on the urinary findings. This has now been accomplished and the rôle of the kidney delineated in a more precise fashion by utilizing the chicken as an experimental animal.[84] Chickens possess a functioning renal-portal circulation.[58] Blood from the leg vein drains directly into the peritubule area of the same side, then enters the systemic circulation via the inferior vena cava, thereby bypassing the glomerulus in the first circulation. Catheterization of the individual ureteral openings in the cloaca permits separate collection of urine from each kidney. Because of this unique situation it is possible to investigate agents which exert effects on tubule cells alone by administering the substance in one leg and comparing urine from the kidney of the infused side with that from the contralateral kidney which serves as a control.[58]

In order to examine the effect of alterations in renal CO_2 tension on acidification, changes in peritubule plasma CO_2 tension were produced by the addition of acid or alkali to saphenous vein blood. The details of one such experiment are summarized in Figure 2. Augmented potassium excre-

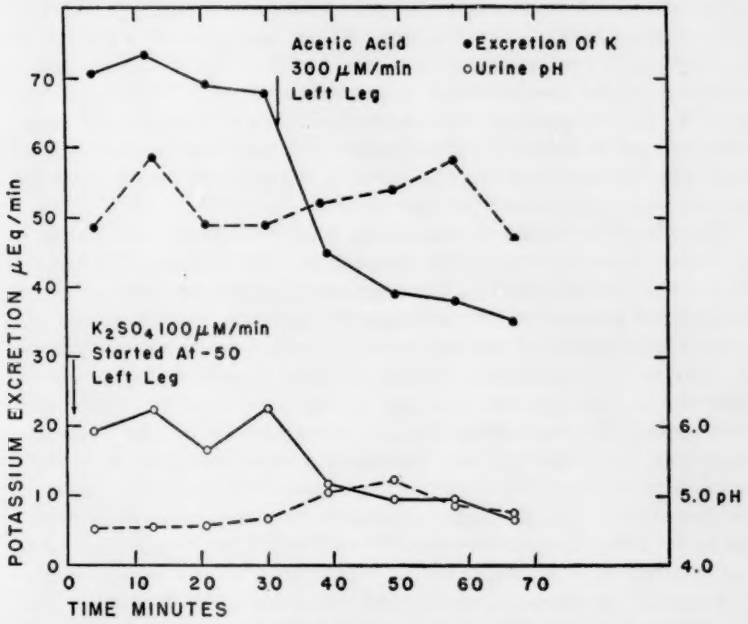

FIG. 2. The effect of elevation of plasma CO_2 tension on urine pH and potassium excretion. Data from the control kidney are illustrated by broken lines, from the experimental kidney by solid lines.

tion and a moderate decrease in hydrogen ion excretion were induced by the administration of potassium sulfate throughout the experiment. Acetic acid was then added to the blood perfusing the left peritubule area. This, by interaction with sodium bicarbonate, elevates plasma CO_2 tension in the immediate area and, since CO_2 diffuses freely, increases intracellular hydrogen ion concentration. The immediate fall in potassium excretion and rise in urine hydrogen ion concentration on that side are apparent. The transformation of acetate to bicarbonate obviates the development of metabolic acidosis. The experiment illustrates two points: (a) elevation of intracellular hydrogen ion and/or CO_2 tension augments hydrogen ion secretion,

and (b) secretion of potassium is inhibited, even in the absence of a fall in the concentration of potassium in the plasma and, presumably, in renal cells. The data are analogous to those observed in experimental and clinical respiratory acidosis in which titratable acid and ammonia excretion rises as excretion of potassium and bicarbonate falls.[16, 17] The converse, reduction of plasma CO_2 tension, was induced by the addition of sodium hydroxide to the saphenous blood. Interaction with carbonic acid produces a diminution in plasma CO_2 tension and resultant interference with acidification. Similar observations have also been made in metabolic alkalosis.[29, 50, 54] In the chicken experiments, it seems clear that the renal excretion of hydrogen and potassium ions can be affected by modifications of intracellular concentration of hydrogen ion alone and that alterations in systemic acid-base balance are not necessary prerequisites for these effects.

The effect of variations in plasma CO_2 tension on urinary acidification is of interest in another regard. The compensatory fall in plasma CO_2 tension in metabolic acidosis resulting from augmented respiratory exchange might be expected to interfere with hydrogen ion secretion. At first glance this appears paradoxical and contrary to bodily needs. Actually, net acidification is augmented in ammonium chloride acidosis, even though the rate of hydrogen ion transfer into urine may be depressed or at least unchanged (see above). The explanation for this phenomenon is obvious when one recognizes that normally a large fraction of secreted hydrogen ion is dissipated in the reabsorption of sodium bicarbonate. This may be in excess of that required for titratable acid and ammonia excretion. In metabolic acidosis, on the other hand, the decreased filtered load of bicarbonate diminishes the requirement of hydrogen ion for reabsorption of this moiety so that hydrogen ion secretion may actually fall with a negligible effect on net acid loss. The reduction in plasma CO_2 tension in this situation, though associated with a fall in the intracellular concentration of hydrogen ion, does not interfere with the regulatory response insofar as total acid-base balance is concerned. The reverse occurs in metabolic alkalosis, i.e., enhanced secretion of hydrogen ions, even though changes in plasma CO_2 tension may be less striking.

THE EFFECT OF THE INTRAVENOUS ADMINISTRATION OF POTASSIUM ON ACIDIFICATION

Berliner, Kennedy, and Orloff pointed out the significance of the reciprocal relationship between potassium and hydrogen ion secretion.[6] They suggested that these ions compete for some portion of the exchange system whereby sodium is reabsorbed. Figure 3 is from an experiment of Orloff and Davidson to illustrate certain features of this competition in the

chicken.[84] It should be noted that administration of potassium in one leg is followed by enhanced excretion of potassium and a rise in urine pH on that side. Presumably the entrance of potassium into tubule cells elevates cell pH, accounting for the observations. In both dog and man the infusion of potassium decreases the excretion of titratable acid and ammonia and increases that of potassium and bicarbonate. The secondary fall in urine pH

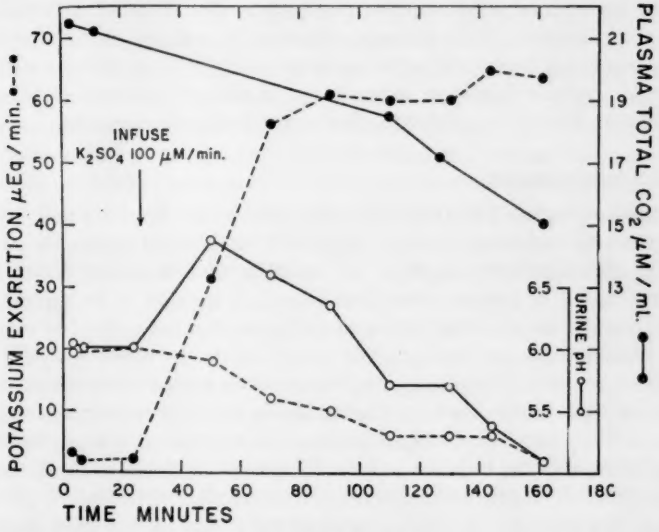

FIG. 3. The effect of intravenous administration of potassium on urine pH, potassium excretion, and plasma total CO_2 concentration. Urine potassium excretion is depicted for the experimental side only. Urine pH on that side is denoted by a solid line, on the control side by a broken line.

noted in the experiment shown in Figure 3 is undoubtedly related to the development of systemic acidosis, as evidenced by the fall in plasma bicarbonate. Acidosis is thought to be due to the extrusion of hydrogen ions from cells in exchange for entering potassium, although loss of alkali in the urine may contribute.[46] As noted earlier, a reduction in filtered load of bicarbonate diminishes the hydrogen ion requirement for bicarbonate reabsorption. Thus aciduria may supervene even when the over-all rate of hydrogen ion secretion is depressed. In the rat, on the other hand, potassium infusions uniformly produce acidosis and aciduria without the interposition of a period of urinary alkalinization.[85] Similar considerations apply to the development of so-called "resistance" to carbonic anhydrase inhibi-

tors. Administration of acetazoleamide, a potent carbonic anhydrase inhibitor, initially induces urinary alkalinization and kaluresis.[6] This may be dependent on an elevation of cell pH alone, though some fall in intracellular potassium concentration might be expected to occur after protracted use of the drug. "Resistance" occurs after prolonged administration as evidenced by aciduria despite metabolic acidosis.[26] This is not due to interference with the action of the drug, as has been suggested, but is related to the depression in filtered load of bicarbonate analogous to that observed after potassium administration. Since carbonic anhydrase is probably not involved in the hydrogen ion transport process, as noted earlier (Fig. 1) even were the enzyme completely inhibited, the uncatalyzed rate of hydration of CO_2 is sufficient for limited intracellular buffering and hydrogen extrusion.

HYPOKALEMIC ALKALOSIS

Potassium depletion is associated with extracellular alkalosis and intracellular acidosis secondary to the entrance of hydrogen and sodium ions into cells in exchange for potassium.[7, 13, 35] Aciduria and decreased potassium excretion noted in potassium-deficient animals is thought to be dependent on reciprocal changes in the hydrogen and potassium ion content of tubule cells. Though it has not been possible to confirm this by direct analyses, as in muscle, this is not surprising, since it would be unlikely that analyses of the whole organ could give valid information as to the chemical composition of the secretory cells.[13] In the light of these considerations, it is apparent that net acid loss from the body is not essential for the development of alkalosis in this situation as originally suggested. The function of the tubule cell is two-fold however: (a) it provides a route for potassium secretion during the development of hypokalemia (unnecessary when produced by gastrointestinal losses) and (b) it serves to perpetuate the alkalosis. Thus the maintenance of plasma bicarbonate at the elevated level induced by the transcellular exchanges indicates that hydrogen ion secretion is enhanced. The compensatory rise in plasma CO_2 tension may potentiate this response.

THE EFFECT OF SODIUM BICARBONATE ADMINISTRATION ON THE EXCRETION OF POTASSIUM

The infusion of sodium bicarbonate induces both kaluresis and alkalinization of the urine. Although superficially this appears to represent competition between hydrogen and potassium ions, intracellular alkalosis being the primary event, evidence in this regard is conflicting. Wallace and Hastings[57] noted no change in cell bicarbonate of muscle when the concentration of bicarbonate in the medium was altered, whereas Anderson and Mudge[1]

noted uptake of bicarbonate and potassium by kidney slices previously depleted of potassium. In contrast, elevation of medium bicarbonate concentration did not affect tissue bicarbonate significantly in slices in which the potassium content was normal. The infusion of sodium bicarbonate into one leg of the chicken does not produce unilateral alkalinization of the urine or kaluresis.[54] This is not necessarily at variance with the hypothesis that cell bicarbonate may rise under these circumstances, nor does it indicate some other cause for the reciprocal changes in electrolyte excretion. The addition of bicarbonate to blood elevates CO_2 tension acutely in the area drained by the vein. Were this sufficient (despite an increase in cell bicarbonate) to augment hydrogen ion secretion, any effect on urine pH and potassium excretion produced by the transfer of bicarbonate into cells might be masked. On the other hand, when bicarbonate is infused into a systemic vein of the chicken, even as in dog and man, kaluresis and alkalinization do occur. Since the rise in CO_2 tension under these circumstances is presumably less, due to the interposition of the lungs, it is possible that intracellular alkalosis may be implicated as the factor responsible for the urinary changes.

AMMONIA EXCRETION

In analyzing the mechanism of ammonia excretion a distinction between those factors which have to do with its intracellular production and those concerned with its transport from cells to urine is essential. In this regard the state of acid-base balance,[18, 44] the availability of precursor amino acids,[20, 38] the integrity of intracellular enzymatic processes[14, 25, 48] all are concerned with production, whereas urine pH,[9, 20, 49 55] urine flow,[38] and the permeability characteristics of the tubule cell membrane influence transport.[38] The concentration of ammonia within cells may be concerned with both processes.[38]

Ammonia is formed within renal tubule cells from precursor amino acids. Van Slyke considered glutamine the major source in the dog, but no confirmation of this observation in other species has been published.[56] Furthermore, little is known about the pathways of deamidation and/or deamination by which urinary ammonia is produced. That slices and homogenates of rat kidney deamidate glutamine to form ammonia is not necessarily proof that this is the major pathway for ammonia production in the intact animal. It is clear, for example, that in the normal dog other amino acids are capable of augmenting ammonia excretion markedly.[20, 38] Transamination may play an important rôle in this process.

The relationship between acid-base balance and ammonia excretion is well known.[18, 49] Thus the daily rate of ammonia excretion increases pro-

gressively in acidotic animals. This may occur in the absence of significant urine pH changes and may represent an increase in cellular production. The mechanism for this adaptive phenomenon has been studied extensively in other species. Davies and Yudkin[14] and others[25, 43, 44] noted increased glutaminase activity in homogenates and slices of renal cortex from acidotic rats. Whether other ammonia-producing enzymes adapt has not been conclusively established, nor has the stimulus to enzyme adaptation been defined. Leonard and Orloff[25] considered it to be related to acidosis since both they and Rector[43] noted in the rat that acetazoleamide-induced extracellular acidosis, despite urine alkalinization, is associated with enhanced ammonia excretion and increased deamidation of glutamine by slices and homogenates of kidney tissue. That this cannot be the only factor is indicated by the observation that hypokalemic alkalosis is also associated with glutaminase activation in this species.*[21]

It may be more reasonable to assume that factors related to intracellular pH and substrate concentration condition the adaptive response. Whether persistent aciduria and consequent enhanced ammonia excretion may under some circumstances stimulate cellular deamidative and deaminative processes is unknown. Leonard and Orloff noted an early rise in ammonia excretion in acidotic rats unassociated with a significant increment in glutaminase activity.[25] They interpreted this as indicative of the presence of other adaptive devices.

The inverse relationship between urine pH and ammonia excretion has been described by a number of investigators.[9, 23, 30, 58] The data are consonant with the view that NH_3, the free base, diffuses out of cells and accumulates in urine as NH_4^+, the extent of accumulation being a function of urine pH. The interposition of a perm-selective membrane freely permeable to NH_3 and less so or not at all to NH_4^+ is essential to this hypothesis.[20] The similar behavior of other weak electrolytes lends credence to this view.[38]

Recently Orloff and Berliner concluded that the accumulation of ammonia in urine reaches equilibrium only in the alkaline range of urine pH.[38] They have considered the kinetics of the process in the following manner. Ammonia diffuses out of cells into urine as a function of the permeability characteristic of the tubule membrane and the concentration of NH_3 in cell and urine respectively. At equilibrium, movement of ammonia in both directions is equal, and the concentration of NH_4^+ will be dependent on

* Acetazoleamide depresses ammonia excretion in dog and man in contrast to its effects in the rat. This throws some doubt on the view that glutaminase adaptation is a general phenomenon in all species.

urine pH.* In essence this is a restatement of the trapping hypothesis of Jacobs, Osterhout, and others who applied it to unicellular systems.[20, 22, 30] In view of the equilibrium nature of the process, it is not surprising that an increase in volume of the receiving fluid (urine) as occurs in diuresis should enhance ammonia excretion. In acid urines, on the other hand, ammonia excretion is independent of urine flow though inversely related to pH. Consequently, it is presumed that equilibrium is not achieved. Under these circumstances it has been postulated that movement of NH_3 is essentially unidirectional and that accumulation of NH_4^+ is limited by maximal rates of production and diffusion of NH_3 out of cells. The most acid urine accumulates the most ammonia by virtue of having been in the acid range over a longer segment of tubule, thereby receiving ammonia at a constant rate for a longer time. It is implicit in this hypothesis that neither acidification nor ammoniation are limited to an isolated segment of tubule, but occur over a variable length of tubule depending on buffer content and rate of hydrogen ion extrusion. In other terms, the most acid urine excreted has had hydrogen ions added to it more rapidly and over a greater length of tubule than any less acid urine of similar buffer content. Either mechanism for the accumulation of NH_4^+, the first a flow-limited equilibrium reaction and the second a production- and diffusion-limited process, is dependent on the differential permeability of the cell membrane to the uncharged and charged members of the buffer pair.

The substitution of ammonium ion for sodium in the urine in chronic acidosis serves to limit the loss of fixed cation from the body. This process was studied in detail by Gamble and by Sartorius.[18, 40] In order to effect the greatest conservation of base, urine pH must remain essentially constant or fall. Thus for each hydrogen ion neutralized by added ammonia, another must exchange for sodium or there will be no net gain of fixed cation by the body. That this does not occur under all circumstances is attested to by results of studies in which precursor amino acids were infused.[38] In these experiments urine pH invariably rose, presumably by virtue of titration with ammonia. Whether the sudden increase in intracellular ammonia production afforded by the added substrate elevates cell pH and interferes with acidification is unclear. Potassium excretion was unaltered, however, a situation not noted in other conditions in which cell pH is presumed to rise. If ammonium ion entered the urine by direct exchange with sodium rather than by diffusion of ammonia, this might conceivably result in interference

* This oversimplification of their analysis does not consider the effect on NH_4^+ accumulation of probable limited permeability of the membrane to NH_4^+; however, this is not crucial to an understanding of the process.

with both potassium and hydrogen secretion. Though there is no direct evidence to controvert this contention, the reproducible relationship between urine pH and ammonia excretion under other circumstances makes it unlikely. Futhermore, Lotspeich and Pitts did not observe urinary alkalinization when they infused amino acids into dogs.[28]

CONCLUSION

Though this was ostensibly a review of the renal regulation of acid-base balance, much has been omitted from consideration. This may be ascribed to ignorance as well as to limitations of space. Much of the discussion has been concerned with the theories of acidification and the factors determining the rate of hydrogen ion secretion. An analysis of base conservation and its rôle in acidosis and alkalosis has been avoided but is implicit throughout. Many of the concepts presented are controversial, the interpretations undoubtedly colored by the prejudices of the writer. Though it may not always be clear where fancy departs from fact, no apologies are offered. John Peters once wrote to the author: "The purpose of this—which I was constrained to publish—was to find out where we stood—and where to go from there. It was just an assemblage of question marks." This in essence was the avowed purpose of this effort.

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THE RÔLE OF SODIUM SALTS AND ADRENAL STEROIDS IN THE PRODUCTION OF HYPOKALEMIC ALKALOSIS§

The administration of adrenal steroids to animals and human subjects on diets containing sodium but little or no potassium is followed by alterations in cellular composition characterized by a loss of potassium which is partially replaced by sodium and the supervention in the extracellular fluid of a metabolic alkalosis.[9] The experiments have been performed principally in rats where the sodium salt used has usually been sodium chloride and the adrenal steroid 11-desoxycorticosterone acetate (DOCA).

The present study of the influence of adrenal steroids on the electrolyte composition of plasma and skeletal muscle was undertaken for the following purposes: (i) to examine the effects of adrenal steroids during fairly complete sodium deprivation; (ii) to determine the influence of the administration of different sodium salts; and (iii) to compare the effect of different adrenal steroids on the pathogenesis of potassium deficiency and alkalosis in the rat.

METHODS AND CALCULATIONS

Male Sprague-Dawley rats weighing 250 to 350 grams were used throughout. When external balances were performed, urine was collected under mineral oil, using toluene and thymol as preservatives. At the end of the experimental period rats were anesthetized by the intraperitoneal injection of Evipal; venous blood was collected anerobically from the inferior vena cava; and muscle samples were removed from the hind legs and trimmed of fat and connective tissue. The samples of muscle were weighed and dried; fat was removed with ether; electrolytes were extracted with 0.75 N nitric acid.[11]

Sodium and potassium were determined on the flame photometer,[8] chloride in serum and muscle extracts by the Hald modification of the open Carius method[14] and in urine

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by the method of Harvey,[9] total proteins by the buiret reaction,[7] nitrogen by the macro-Kjeldahl,[24] and the CO_2 content of serum by the method of Van Slyke and Neill.[34] Muscle water was determined as the loss of weight after drying at 100° C. for three days. Serum water, $[W]_s$, was estimated from the concentration of serum protein, $[P]_s$, according to the formula:

$$0.985 - 0.00745\ [P]_s = [W]_s$$

The concentrations in extracellular fluid of sodium, $[Na]_e$, and chloride, $[Cl]_e$, were estimated from their serum concentrations, $[Na]_s$ and $[Cl]_s$, the water content of serum, $[W]_s$, and an average Donnan factor of 0.95:

$$\frac{[Na]_s \times 0.95}{[W]_s} = [Na]_e$$

$$\frac{[Cl]_s}{[W]_s \times 0.95} = [Cl]_e$$

The concentration of potassium was assumed to be the same in serum and extracellular fluid. The amount of intracellular sodium per 100 grams of fat-free dry tissue, $(Na)_i$ was estimated according to the method of Darrow and his associates[6]:

$$\frac{(Cl)_m - 1}{[Cl]_e} = (H_2O)_e$$

$$(Na)_m - (H_2O)_e\ [Na]_e = (Na)_i$$

in which $(Cl)_m$ and $(Na)_m$ are total muscle chloride and sodium, respectively, per 100 grams of fat-free dry tissue (FFDT) and $(H_2O)_e$ is the extracellular water per 100 grams FFDT. The amount of intracellular water per 100 grams FFDT was calculated according to the equation:

$$(H_2O)_m - (H_2O)_e = (H_2O)_i$$

where $(H_2O)_m$ is the total muscle water per 100 grams FFDT.

RESULTS

The data are presented in Tables 1-6 as the mean and standard deviation of each group.

1. *Effect of DOCA on exchanges of electrolytes and water in rats maintained on a low sodium diet containing minimal or large amounts of potassium.* Four groups of rats maintained on a diet low in sodium (0.26 mEq./100 gm. diet) and supplemented with potassium in minimal (4.6 mEq./100 gm. diet) or large (41.4 mEq./100 gm. diet) amounts were injected with 5 mg. DOCA daily and sacrificed after two weeks. The minimal potassium intake was estimated to be sufficient just barely to prevent a potassium deficit owing to an inadequate intake. Should DOCA accelerate potassium excretion by a direct action, a negative balance would then be easily demonstrable. The cumulative balances are charted in Table 1. The administration of DOCA significantly reduced the excretion of sodium as compared to their

TABLE 1. EXTERNAL BALANCES FOR TWO WEEKS OF RATS ON MINIMAL AND HIGH POTASSIUM DIETS WITH AND WITHOUT DOCA

Group and no. of rats	*Treatment*	*Intake*				*Urine output*				*External balance*			
		Na	*K*	*Cl*	*N*	*Na*	*K*	*Cl*	*N*	*Na*	*K*	*Cl*	*N*
		mEq.	*mEq.*	*mEq.*	*gms.*	*mEq.*	*mEq.*	*mEq.*	*gms.*	*mEq.*	*mEq.*	*mEq.*	*gms.*
B; 4	Minimal K diet	0.21 ±0.07	3.66 ±0.40	0.68 ±0.07	0.51 ±0.06	0.49 ±0.23	3.20 ±0.71	1.32 ±0.41	0.80 ±0.55	−0.28 ±0.23	+0.46 ±0.71	−0.64 ±0.40	−0.29 ±0.53
C; 4	Minimal K diet + DOCA	0.18 ±0.03	3.11 ±0.49	0.58 ±0.09	0.46 ±0.07	0.19 ±0.13	2.48 ±0.84	0.90 ±0.38	0.46 ±0.13	−0.01 ±0.12	+0.63 ±0.86	−0.32 ±0.32	−0.02 ±0.12
D; 4	High K diet	0.21 ±.04	32.9 ±7.0	0.68 ±0.15	0.45 ±0.09	0.43 ±0.08	15.7 ±3.4	0.87 ±0.16	0.45 ±0.03	−0.22 ±0.11	+17.3 ±4.7	−0.18 ±0.09	+0.001 ±0.011
E; 4	High K diet + DOCA	0.21 ±0.10	32.8 ±9.2	0.68 ±0.19	0.44 ±0.13	0.23 ±0.05	13.8 ±2.6	0.55 ±0.04	0.38 ±0.15	−0.02 ±0.07	+19.1 ±7.3	+0.13 ±0.16	+0.06 ±0.14

controls irrespective of whether minimal potassium (Group C) or high potassium (Group E) diet was given. However, since the intake of sodium was very low, the absolute amount of sodium retained was trivial. Potassium excretion was not accelerated by DOCA whether the potassium intake was low or high.

Analysis of serum and muscle (Table 2) revealed no significant influence of DOCA on electrolyte composition. Total muscle water was not significantly altered by the administration of DOCA. Using chloride as a reference for the determination of the extracellular space of muscle, it can be concluded that since muscle chloride and total muscle water were not significantly altered, the intracellular and extracellular spaces of muscle remained constant.

From the standpoint of a balance study, as well as the direct analysis of muscle, it would appear that DOCA has no significant effect on internal or external exchanges of potassium, sodium, and water if sodium salts are rigidly excluded from the diet.

2. *Effect of DOCA on exchanges of electrolytes and water in rats maintained on a low potassium diet supplemented with different sodium salts.* To examine the effects of DOCA with and without the addition of different sodium salts, eight groups of rats were fed a standard electrolyte-deficient diet (EDD), complete in all respects but deficient in sodium, potassium, and chloride. Equivalent amounts of sodium, either as sodium chloride or as sodium phosphate (pH 7.4), were added to the diet of some groups. The electrolyte composition of the diet is given in Table 3. Groups E and H (Tables 4 and 5) received injections of 5 mg. of DOCA daily.

The effect of supplements of NaCl and $Na\text{-}PO_4$ to an EDD diet were examined in rats after a seven-day period (Table 4). No significant change in serum bicarbonate or intracellular sodium and potassium resulted from the addition of sodium chloride to EDD (Table 4, Group B). Rats given EDD + $Na\text{-}PO_4$ (Table 4, Group C) developed a rise in the serum bicarbonate while muscle potassium fell and intracellular sodium rose.

Rats maintained on EDD alone for 36 days (Table 4, Group D) manifested little change in electrolyte composition as compared with the group maintained on the same diet for only seven days (Group A): the slight rise in mean serum bicarbonate (from 30.5 mEq./L in Group A to 34.1 mEq./L in Group D) was not significant; muscle potassium and intracellular sodium were virtually the same in the two groups. The injection of 5 mg. of DOCA to rats receiving EDD only (Group E) did not significantly alter the serum bicarbonate or the electrolyte composition of muscle. The addition of sodium chloride (Group F) or sodium phosphate (Group G) to EDD resulted in only minor changes in serum bicarbonate and muscle

TABLE 2. SERUM AND MUSCLE ANALYSES OF RATS ON MINIMAL AND HIGH POTASSIUM DIETS WITH AND WITHOUT DOCA

Group and no. of rats	Treatment	Duration	Serum concentration					Muscle composition per 100 grams of fat-free dry tissue						
			Cl	*Na*	*K*	*Total proteins*	H_2O	*N*	H_2O	*Cl*	*Na*	*K*	*Na*$_i$	*K/N*
		days	*mEq./liter*	*mEq./liter*	*mEq./liter*	*gms./liter*	*gms./liter*	*gms.*	*gms.*	*mEq.*	*mEq.*	*mEq.*	*mEq.*	
A; 5	Minimal K diet	5	100.4 ±1.1	146.8 ±0.5	4.78 ±0.24			15.24 ±0.13	320 ±3	5.43 ±0.42	8.92 ±0.88	46.7 ±0.8	3.07 ±0.37	3.06 ±0.04
B; 4	Minimal K diet	14	98.6 ±1.0	141.7 ±1.6	5.63 ±0.40	54.2 ±1.7	945 ±2	14.27 ±0.20	323 ±3	5.45 ±0.20	8.10 ±0.32	47.2 ±0.9	2.34 ±0.21	3.31 ±0.10
C; 4	Minimal K diet + DOCA	14	99.4 ±0.7	144.7 ±0.9	5.54 ±0.37	49.2 ±1.1	949 ±8	14.54 ±0.37	320 ±5	5.35 ±0.28	8.51 ±0.59	46.8 ±1.1	2.77 ±0.81	3.22 ±0.15
D; 4	High K diet	14	98.6 ±0.9	143.8 ±0.2	5.61 ±0.08	52.0 ±3.7	947 ±3	14.31 ±0.12	325 ±4	4.75 ±0.20	7.52 ±0.70	48.8 ±1.3	2.59 ±0.45	3.41 ±0.10
E; 4	High K diet + DOCA	14	99.0 ±1.4	143.9 ±0.4	5.68 ±0.26	49.5 ±1.8	949 ±2	14.56 ±0.37	319 ±5	5.11 ±0.34	8.88 ±0.65	47.0 ±0.7	3.19 ±0.33	3.23 ±0.11

composition. The most striking results occurred when DOCA was given with sodium phosphate for a period of only 13 days (Group H). The magnitude of the hypochloremic alkalosis and potassium deficiency far exceeded that obtained in other studies where rats were given DOCA and low potassium diets but where the added sodium salt was sodium chloride.[4, 6, 13]

Despite the striking changes in the intracellular electrolyte composition of muscle cells in those rats given sodium salts and DOCA, the volume of intracellular fluid remained remarkably constant (Table 5).

To estimate the total cation concentration of intracellular fluid, the sum of the concentrations of sodium and potassium was used. This would seem

TABLE 3. COMPOSITION OF DIET PER 100 GRAMS

*Type of diet**	*Na*	*Cl*	*K*	*N*
	mEq.	*mEq.*	*mEq.*	*gms.*
EDD	0.67	0.37	0.13	1.90
EDD+Na—P†	44	1.95	0.14	1.95
EDD+NaCl	44	44	0.17	1.98

* EDD = electrolyte-deficient diet—a basic diet deficient in Na, Cl, and K.
† A mixture of Na_2HPO_4 and NaH_2PO_4 of pH 7.4 was added to diet.

to give a reasonably good approximation of the behavior of total cation, inasmuch as intracellular magnesium (which normally amounts to about 34 mEq./L) has been demonstrated to remain unchanged in potassium deficiency.[6] In Table 5, the mean values for volume and total cation concentration of the extracellular and intracellular compartments of muscle have been recorded.

Despite the constancy of the volume of intracellular fluid, there is a tendency for the total cation concentration to fall, particularly in the rats given sodium phosphate and DOCA. The significance of this fall in total cation concentration in this group is enhanced by the fact that the total cation concentration of extracellular fluid showed no commensurate reduction, but instead increased slightly (3 mEq./L). This disparity between the changes in the concentration of total cation in extracellular and intracellular fluids on the one hand and the constancy of intracellular water on the other hand should not necessarily be interpreted to mean that the osmotic pressure of the intracellular and extracellular phases differ. Such a discrepancy could result, as Cotlove and his associates have pointed out,[5] from an activation of osmotically inactive intracellular cations, an increase in the

TABLE 4. THE EFFECTS OF DIFFERENT Na SALTS AND DOCA ON THE SERUM AND MUSCLE COMPOSITION OF RATS MAINTAINED ON AN ELECTROLYTE-DEFICIENT DIET

Group and no. of rats	Treatment	Duration	Serum concentration							Muscle composition per 100 grams fat-free dry tissue							Body weight change
			CO_2	Cl	Na	K	R*	Total proteins	H_2O†	N	H_2O	Cl	Na	K	Na_i‡	K/N	
		days	mEq./liter	mEq./liter	mEq./liter	mEq./liter	mEq./liter	gms./liter	gms./liter	gms.	gms.	mEq.	mEq.	mEq.	mEq.		gms.
A; 4	EDD	7	30.5	96.8	148.3	5.00	21.4	61.4	939	15.05	308	4.77	10.75	42.9	5.33	2.85	
			±1.4	±1.9	±0.5	±0.08	±2.1	±2.7	±2	±0.18	±4	±0.16	±0.37	±1.5	±0.29	±0.12	
B; 5	EDD + NaCl	7	31.4	100.9	150.7	4.43	18.4	55.8	943	15.20	325	5.94	12.72	43.2	6.07	2.85	
			±2.1	±0.8	±0.8	±0.13	±1.7	±2.6	±2	±0.38	±4	±0.29	±1.00	±1.0	±1.12	±0.13	
C; 4	EDD + Na — P	7	35.3	90.6	149.6	3.71	23.5	61.8	939	15.19	312	5.04	17.00	39.2	10.95	2.58	
			±2.9	±2.8	±1.2	±0.31	±2.6	±3.0	±2	±0.16	±2	±0.36	±1.95	±1.1	±1.50	±0.08	
D; 7	EDD	36	34.1	86.5	146.9	4.36	26.4	67.1	935	15.33	316	4.99	12.90	42.6	6.78	2.78	—27
			±2.9	±4.5	±1.4	±0.30	±1.8	±2.8	±2	±0.38	±4	±0.65	±1.39	±0.8	±1.60	±0.10	±13
E; 7	EDD + DOCA	35	34.2	92.1	147.7	4.14	22.0	60.1	940	15.34	317	5.14	13.80	40.1	7.80	2.61	—11
			±0.9	±2.3	±0.9	±0.66	±2.5	±3.3	±3	±0.10	±6	±0.44	±1.16	±1.6	±0.57	±0.13	±11
F; 6	EDD + NaCl	15	36.3	91.9	150.0	3.49	23.0	56.4	943	14.98	325	5.74	16.67	40.1	9.70	2.67	+3
			±3.4	±5.8	±1.9	±1.16	±5.1	±5.3	±4	±0.30	±10	±0.77	±2.30	±2.4	±1.77	±0.17	±32
G; 7	EDD + Na — P	16	36.9	88.0	149.7	3.62	24.8	55.9	943	15.09	330	5.63	17.80	38.0	10.68	2.52	—2
			±1.4	±3.0	±0.8	±0.15	±2.5	±2.7	±2	±0.44	±17	±0.41	±1.58	±0.9	±1.09	±0.05	±5
H; 6	EDD + Na — P + DOCA	13	45.3	76.2	153.0	3.25	31.5	44.9	952	15.52	328	5.81	22.35	32.7	13.69	2.12	—13
			±2.8	±4.1	±1.2	±0.19	±2.6	±2.5	±2	±0.62	±6	±1.24	±1.16	±0.6	±1.19	±0.12	±10

* $[R] = [Na] - ([Cl] + [CO_2])$.

† Estimated from the concentration of total proteins, $[P]_s$, according to the equation $[W]_s = 0.985 - 0.00745\ [P]_s$.

‡ Na_i = intracellular sodium per 100 grams of fat-free dry tissues.

TABLE 5. VOLUME AND CONCENTRATIONS PER 100 GRAMS OF FAT-FREE DRY TISSUE OF INTRACELLULAR AND EXTRACELLULAR CONSTITUENTS OF RATS LISTED IN TABLE 4

Group and no. of rats	*Treatment*	*Duration*	*Extracellular space*					*Intracellular space*			
			Volume	*Cl*	*Na*	*K*	*Na + K*	*Volume*	*Na*	*K*	*Na + K*
		days	*gms.*	*mEq./liter*	*mEq./liter*	*mEq./liter*	*mEq./liter*	*gms.*	*mEq./liter*	*mEq./liter*	*mEq./liter*
A; 4	EDD	7	34.8	108.5	150.0	5.00	155.0	274	20.2	156.6	176.8
			±2.1	±2.2	±0.6	±0.08	±0.7	±4	±1.3	±3.8	±3.8
B; 5	EDD + NaCl	7	43.8	112.6	151.8	4.43	156.3	281	23.1	153.7	176.8
			±3.3	±1.0	±0.7	±0.13	±0.8	±2	±2.7	±3.9	±3.9
C; 4	EDD + Na — P	7	40.0	101.5	151.3	3.71	155.0	272	40.3	143.9	184.2
			±4.6	±2.3	±1.1	±0.31	±1.3	±3	±5.8	±3.8	±4.3
D; 7	EDD	36	40.9	97.4	149.3	4.36	153.3	275	24.6	154.8	179.4
			±5.1	±5.1	±1.5	±0.30	±1.2	±4	±5.8	±3.8	±3.3
E; 7	EDD + DOCA	35	40.2	103.1	149.2	4.14	153.4	277	28.2	144.6	172.8
			±4.9	±2.4	±0.8	±0.66	±0.8	±7	±2.1	±6.7	±6.0
F; 6	EDD + NaCl	15	46.2	102.6	151.1	3.49	154.6	279	34.8	143.8	178.6
			±6.5	±6.2	±2.0	±1.16	±1.8	±5	±6.1	±10.1	±8.5
G; 7	EDD + Na — P	16	47.1	98.2	150.8	3.62	154.4	283	37.8	134.5	172.3
			±4.3	±3.5	±0.8	±0.15	±0.8	±16	±3.7	±8.3	±9.2
H; 6	EDD + Na — P + DOCA	13	56.7	84.4	152.8	3.25	156.0	271	50.5	120.9	171.4
			±13.1	±4.4	±1.3	±0.19	±1.3	±12	±3.6	±7.0	±7.2

intracellular concentrations of osmotically active anions, or an increase in the concentration of intracellular nondiffusible neutral molecules.

3. *Effect of DOCA on potassium excretion and acid-base balance during rigid sodium restriction in human subjects.* Two human subjects were maintained for prolonged periods of time on a diet containing 6 mM. of sodium chloride daily and given daily injections of 20 mg. DOCA. The results in

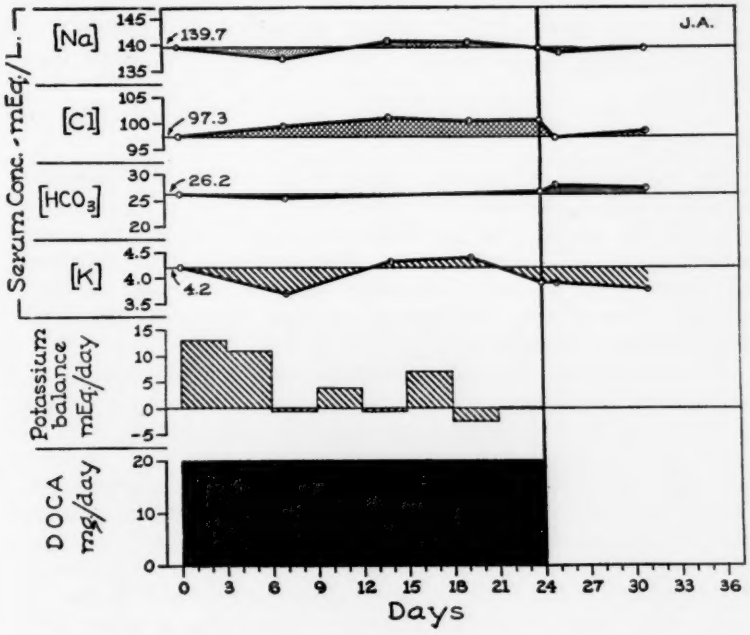

FIG. 1. The effect of DOCA on serum composition and potassium balance during salt restriction.

one of these subjects are plotted in Figure 1. As in the rat experiments, no acceleration of potassium excretion or rise in bicarbonate transpired after 24 days on this regimen. Similar observations have been made in man by Relman and Schwartz.[15]

4. *Effect of cortisone on exchanges of electrolytes and water in rats maintained on a low potassium diet supplemented with different sodium salts.* The injection of cortisone in doses of 5 mg. per kilogram of body weight tended to lower the serum bicarbonate toward normal, reduce intracellular

sodium, and increase muscle potassium. This pattern was observed even in those rats given sodium phosphate (Table 6). While the changes were not always significant, mean values in the cortisone-treated animals were more nearly normal in every instance. These changes are especially noteworthy when contrasted with the effects of DOCA. In rats given sodium phosphate and DOCA the mean values for serum bicarbonate, muscle potassium, and intracellular sodium were 45.3 mEq./L, 32.7 mEq. per 100 grams FFDT and 13.7 mEq. per 100 grams FFDT, respectively. In the cortisone group given sodium phosphate, the mean values were 31.7 for serum bicarbonate, 44.1 for muscle potassium, and 7.2 for intracellular sodium.

DISCUSSION

The results of these experiments indicate that in the absence of exogenous supplies of sodium salts, DOCA has no effect on the electrolyte composition of serum and muscle. The simultaneous administration of DOCA and sodium salts to rats on a low potassium diet produces potassium deficiency and hypochloremic alkalosis. These disturbances will also result, although to a lesser extent, when sodium salts are given without DOCA if the potassium intake is restricted.

It seems likely that DOCA and exogenous sodium salts produce a potassium diuresis principally as a result of exchanges in the renal tubules rather than as a consequence of pre-renal effects. The administration of DOCA has no effect on the serum or muscle potassium in the nephrectomized animal.[21, 22] The administration of large amounts of sodium chloride does not accelerate potassium excretion in patients with Addison's disease whose renal tubules do not reabsorb increments of the administered sodium.[8] The elevated serum potassium that follows the injection of large amounts of saline to nephrectomized animals seems better correlated with the dilution acidosis which results rather than with any direct exchange of extracellular sodium for intracellular potassium.[10] Berliner, Kennedy, and Orloff[1] hypothesized that potassium secretion in the distal tubules was accompanied by the exchange of sodium in the tubular lumen for potassium in the tubular cells. This hypothesis would explain the marked potassium diuresis produced by the simultaneous administration of DOCA and sodium salts: exogenous sodium is required to furnish large amounts of this cation at the exchange site; DOCA is required to promote the reabsorption of increments of sodium in exchange for potassium.

It is not clear why sodium phosphate is so much more effective than is sodium chloride in accelerating potassium loss. The phosphate ion per se is not responsible for this effect, inasmuch as sodium sulfate is equally ef-

TABLE 6. THE EFFECTS OF DIFFERENT Na SALTS AND CORTISONE ON THE SERUM AND MUSCLE COMPOSITION OF RATS MAINTAINED ON AN ELECTROLYTE-DEFICIENT DIET

Group and no. of rats	*Treatment*	*Duration*	*Serum concentration*							*Muscle composition per 100 grams fat-free dry tissue*							*Body weight change*
			CO_2	*Cl*	*Na*	*K*	*R*	*Total protein*	*H_2O*	*N*	*H_2O*	*Cl*	*Na*	*K*	*Nai*	*K/N*	
		days	*mEq./liter*	*mEq./liter*	*mEq./liter*	*mEq./liter*	*mEq./liter*	*gms./liter*	*gms./liter*	*gms.*	*gms.*	*mEq.*	*mEq.*	*mEq.*	*mEq.*		*gms.*
A; 7	EDD	36	34.1	86.5	146.9	4.36	26.4	67.1	935	15.33	316	4.99	12.90	42.6	6.78	2.78	—27
			±2.9	±4.5	±1.4	±0.30	±1.8	±2.8	±2	±0.38	±4	±0.65	±1.39	±0.8	±1.60	±0.10	±13
B; 8	EDD + Cortisone	35	32.5	95.7	147.7	4.68	20.6	59.9	940	15.32	312	5.11	9.76	43.8	3.99	2.86	—52
			±1.4	±3.2	±1.4	±0.58	±2.9	±3.5	±3	±0.60	±4	±0.42	±1.45	±0.6	±1.06	±0.12	±10
C; 6	EDD + NaCl	15	36.3	91.9	150.0	3.49	23.0	56.4	943	14.98	325	5.74	16.67	40.1	9.70	2.67	+3
			±3.4	±5.8	±1.9	±1.16	±5.1	±5.3	±4	±0.30	±10	±0.77	±2.30	±2.4	±1.77	±0.17	±32
D; 7	EDD + NaCl + Cortisone	15	31.1	95.1	151.8	4.76	25.7	63.8	937	14.52	318	5.78	12.22	45.06	5.34	3.11	—44
			±3.1	±1.3	±1.9	±0.91	±4.0	±8.1	±6	±0.77	±4	±0.37	±1.40	±1.09	±0.99	±0.16	±12
E; 7	EDD + Na — P	16	36.9	88.0	149.7	3.62	24.8	55.9	943	15.09	330	5.63	17.80	38.0	10.68	2.52	—2
			±1.4	±3.0	±0.8	±0.15	±2.5	±2.7	±2	±0.44	±17	±0.41	±1.58	±0.9	±1.09	±0.05	±5
F; 6	EDD + Na — P + Cortisone	15	31.7	95.5	149.2	4.10	22.0	60.4	940	14.80	313	5.09	13.00	44.1	7.24	2.98	—53
			±4.8	±3.6	±1.7	±0.23	±4.2	±2.3	±2	±0.49	±2	±0.34	±1.93	±2.4	±2.18	±0.09	±16

fective. A second hypothesis would account for the difference by assuming that most of the exogenous sodium chloride is reabsorbed in the proximal tubules, so that comparatively small amounts reach the distal tubular exchange site, whereas most of the sodium phosphate or sodium sulfate would pass unreabsorbed through the proximal tubule, thereby resulting in the delivery of comparatively large amounts of sodium to the distal tubule where it can be reabsorbed in exchange for potassium. This hypothesis cannot satisfactorily account for the data in this study. The diets contained 44 mEq. of sodium, as sodium chloride or sodium phosphate, per 100 grams. Since the rats consumed about 30 grams of diet daily, about 180 mM. of sodium chloride were ingested in a two-week period. Only a comparatively small proportion of the ingested sodium was retained in the body; the rest must have traversed the distal tubule to be excreted into the urine. It seems likely that the presence of an unreabsorbed anion potentiates the exchange of sodium for potassium in some as yet unknown manner.

The relationship among the changes in the volume and composition of serum and muscle cells is examined in Table 7. The changes in the concentrations of serum electrolytes, as muscle potassium is progressively depleted, consist of a rise in bicarbonate, a fall in chloride, a fall in potassium, a rise in sodium, and an expansion of fixed anions (determined as the difference between sodium and the sum of bicarbonate plus chloride).

The correlation between the fall in muscle potassium and the rise in serum bicarbonate was excellent (–0.80 for the group as a whole, –0.91 for the group given sodium phosphate and DOCA). Two factors appear to be responsible for the alkalosis which develops under the conditions of these experiments: (i) a transfer of hydrogen ions into muscle cells in exchange for potassium;[2, 4, 15, 16] (ii) an accelerated renal excretion of acid, largely in the form of ammonium, apparently resulting from an enhanced exchange in the kidneys of sodium in the tubular lumen for hydrogen in the tubular cell.[18] It should be emphasized that the excellent correlation of potassium deficiency and alkalosis, observed in these experiments, where sodium salts and DOCA were responsible for the derangements, may not obtain when potassium deficiency is produced by other means. It is not unusual clinically to observe severe degrees of potassium deficiency in patients who are deprived of this electrolyte where the serum bicarbonate remains normal.

The rise in the serum bicarbonate as potassium deficiency develops is associated with a fall in the concentration of serum chloride (Fig. 2).

The cause of the hypochloremia is not clear. It is not a consequence of chloride deprivation, inasmuch as hypochloremia can supervene despite generous amounts of chloride in the diet. It is possible that the expansion of the extracellular volume with sodium bicarbonate, as alkalosis develops,

TABLE 7. STATISTICAL ANALYSIS OF THE RELATIONS AMONG SERUM AND MUSCLE CONSTITUENTS OF RATS LISTED IN TABLE 4

Groups; no. of rats	*Mean and standard deviation of serum or muscle constituent*	*Regression equations with standard error of estimate*	*Correlation coefficient*
All groups of Table IV	$[Na]_s = 149.5 \pm 2.2$ mEq.	$[Na]_s = 165.1 - 0.39Km \pm 3.8$	
		$Km = 191.3 - 1.01[Na]_s \pm 6.0$	−0.63
	$[K]_s = 3.93 \pm 0.67$ mEq.	$[K]_s = -1.22 + 0.13Km \pm 0.49$	
	$[HCO_3]_s = 35.7 \pm 5.1$ mEq.	$Km = 25.6 + 3.55[K]_s \pm 2.5$	0.68
46 rats	$[Cl]_s = 89.2 \pm 7.5$ mEq.	$[Cl]_s = 28.3 + 1.54Km \pm 5.2$	
	$[Cl]_{ECW} = 99.8 \pm 8.5$ mEq.	$Km = 9.5 + 0.34[Cl]_s \pm 2.4$	0.72
	$Km = 39.6 \pm 3.5$ mEq.	$[Cl]_{ECW_m} = 28.2 + 1.81Km \pm 5.7$	
	$Na_i = 9.09 \pm 2.86$ mEq.	$Km = 9.2 + 0.31[Cl]_{ECW_m} \pm 2.3$	0.74
	$ECW_m = 44.3 \pm 8.2$ gms.	$[HCO_3]_s = 82.0 - 1.17Km \pm 3.0$	
		$Km = 59.4 - 0.56[HCO_3]_s \pm 2.1$	−0.80
		$[Cl]_s = 20.4 + 0.64ECW_m \pm 5.3$	
		$ECW_m = -25.4 + 0.78[Cl]_s \pm 5.8$	−0.71
		$Km = 49.6 - 1.10Na_i \pm 1.5$	
		$Na_i = 38.2 - 0.74Km \pm 1.3$	−0.90
Na — P Group (C, G, H); 17 rats	$[Na]_s = 150.1 \pm 1.9$ mEq.	$[Na]_s = 170.4 - 0.54Km \pm 1.0$	
	$[Cl]_s = 84.5 \pm 6.9$ mEq.	$Km = 228.6 - 1.27[Na]_s \pm 1.6$	−0.83
		$[Cl]_s = 8.21 + 2.10Km \pm 3.4$	
	$[HCO_3]_s = 39.5 \pm 4.8$ mEq.	$Km = 5.82 + 0.36[Cl]_s \pm 1.4$	0.87
	$Km = 36.4 \pm 2.9$ mEq.	$[HCO_3]_s = 91.9 - 1.44Km \pm 2.0$	
		$Km = 59.0 - 0.57[HCO_3]_s \pm 1.2$	−0.91

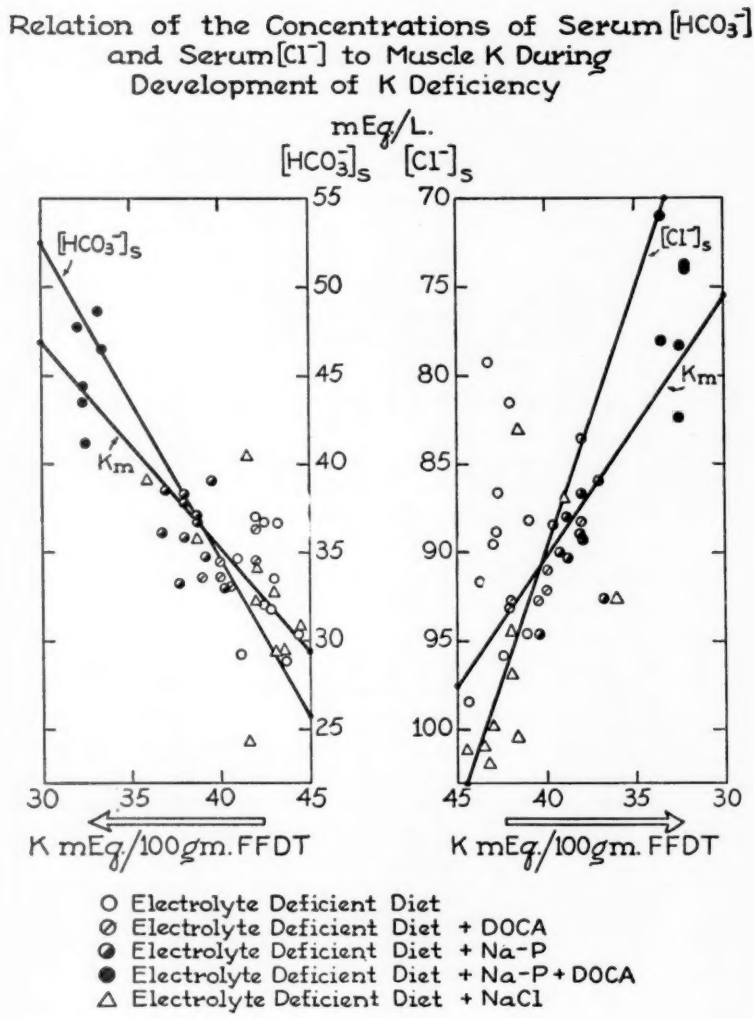
Relation of the Concentrations of Serum [HCO3-] and Serum [Cl-] to Muscle K During Development of K Deficiency
mEq/L.
[HCO3-]s
[Cl-]s
[HCO3-]s
Km
[Cl-]s
Km
K mEq/100gm. FFDT
K mEq/100gm. FFDT
○ Electrolyte Deficient Diet
⊘ Electrolyte Deficient Diet + DOCA
◪ Electrolyte Deficient Diet + Na-P
● Electrolyte Deficient Diet + Na-P + DOCA
△ Electrolyte Deficient Diet + NaCl

FIG. 2.

results in a depression of the concentration of serum chloride by dilution. There is a good correlation among the changes in serum chloride, serum bicarbonate, and extracellular space of muscle as potassium deficiency develops (Table 7). The correlation is especially good in the rats given sodium phosphate and DOCA, where the expansion of extracellular space is a consequence of the accession principally of sodium bicarbonate. These animals, having no access to chloride, cannot retain sodium chloride, a process which could mask the magnitude of the hypochloremia attributable to dilution of the extracellular fluid with sodium bicarbonate. That dilution, rather than loss of chloride into the urine as ammonium chloride, is responsible for the hypochloremia receives support from the observation of Schwartz and his associates[17] that total body chloride is normal during hypokalemic alkalosis in the rat despite depressed serum concentrations.

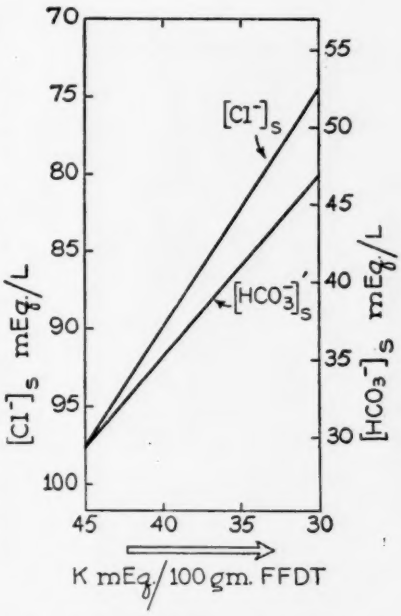

FIG. 3. Relation between the rise in serum $[HCO_3^-]$ and fall in serum $[Cl^-]$ during development of K deficiency.

In Figure 3 the changes in serum bicarbonate and chloride are compared. The vertical axis is so adjusted that the normal concentrations of serum bicarbonate and chloride fall on a single point. The straight lines for chloride and bicarbonate are taken from the regression data in Table 7. It is apparent that in these studies (which include the groups given sodium phosphate) the concentration of serum chloride fell more than bicarbonate rose. The reason for this appears to be an expansion of fixed anions $[R^-]$, which transpires as potassium deficiency develops (Fig. 4). Part of the increase in $[R^-]$ is associated with the development of a mild hypernatremia; but fixed anions also expand at the expense of bicarbonate. Some of the increase in fixed anions can be attributed to enhanced binding of cation during alkalosis. If it is assumed that pH changes observed by others in hypokalemic alkalosis apply in these studies as well, then the

expansion of fixed anions is too great to be attributed solely to this process. In addition there was probably an accession of undetermined anions to the extracellular fluid.

The fall in serum potassium was only moderately well correlated with cellular potassium deficiency (Fig. 5). It is particularly noteworthy that rats simply deprived of potassium (EDD alone) tended in some instances to have a normal serum potassium despite the development of cellular deficits. This is in accord with the clinical observations that cellular deficits can occur without being reflected by hypokalemia.

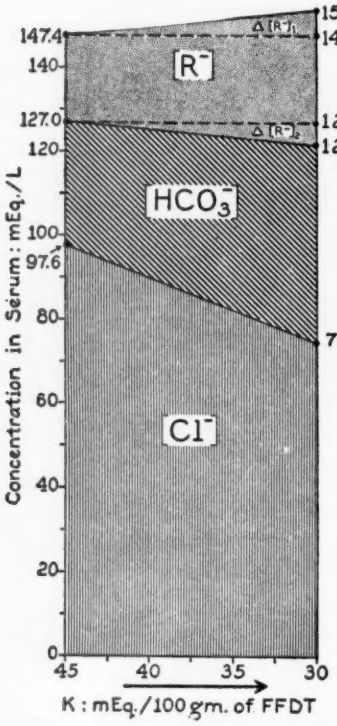

FIG. 4. The relation of the concentrations of serum electrolytes to decreases in muscle potassium.

Unlike the experiments with DOCA and sodium salts discussed above, where hypochloremic alkalosis was regularly produced, the administration of cortisone, even with sodium phosphate, resulted in an amelioration of the process: in the serum the concentrations of bicarbonate fell, while chloride and potassium rose, while in muscle, intracellular sodium fell and potassium rose (Table 6). The weight loss of cortisone-treated animals was marked. Inasmuch as this weight loss involved the breakdown of tissues with the release of their potassium content, it seems reasonable to assume that this process was responsible for the correction of hypokalemic alkalosis. The release of potassium in association with tissue breakdown could furnish an endogenous infusion of this cation which would then be available to replenish the deficit of intact cells, thereby correcting the intracellular and extracellular electrolyte derangements in the same manner that would be achieved by the administration of exogenous potassium salts. In a similar way, potassium-deficient dogs which are nephrectomized replenish their depleted intracellular stores with the potassium released by tissue

breakdown.[13] Unlike the rat, the administration of cortisone to man often results in hypokalemic alkalosis but not in its correction. This difference in response to cortisone could be the result of the propensity of the hormone in

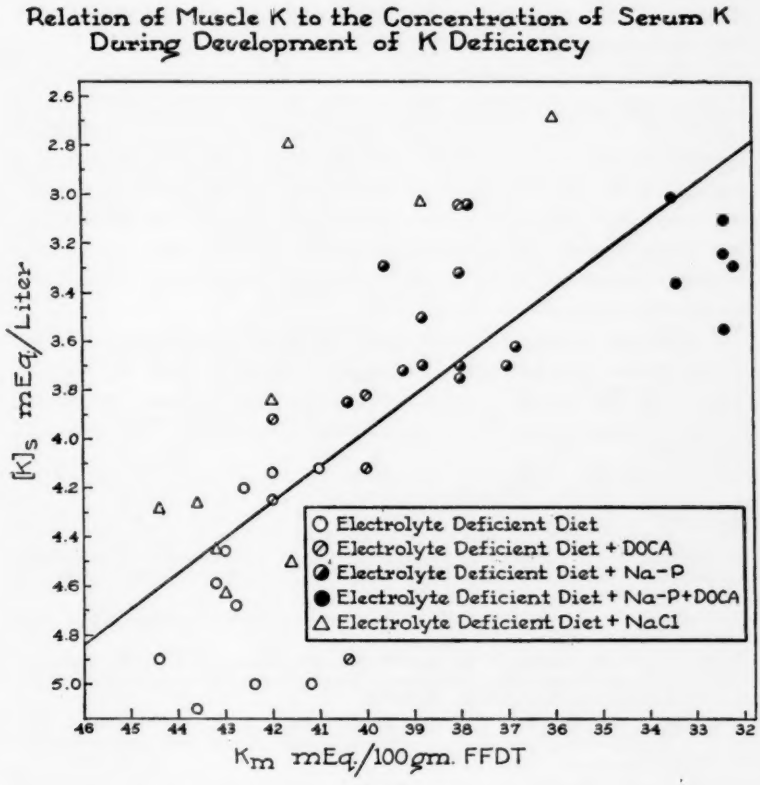

FIG. 5.

the rat to stimulate appetite (and thereby augment potassium intake) to a greater extent, to augment the renal tubular sodium-potassium exchange less powerfully, or to elicit more tissue breakdown.

SUMMARY

1. The administration of DOCA to rats on low or high potassium intakes had no effect on exchanges of potassium, acid-base balance, or the volume

of extracellular and intracellular fluid of muscle if sodium was rigidly restricted from the diet.

2. The addition of sodium salts to a low potassium diet, particularly if DOCA was simultaneously injected, resulted in potassium deficiency and alkalosis. Sodium phosphate (pH 7.4) was much more effective in producing hypokalemic alkalosis than sodium chloride.

3. The changes in concentrations of extracellular electrolytes during potassium deficiency produced in this manner were: a rise in bicarbonate, a fall in chloride and potassium, a rise in sodium, and a rise in unidentified fixed anions. It is suggested that hypochloremia may be, in part at least, a consequence of the dilution of the extracellular space with sodium bicarbonate and water. Changes in intracellular composition were characterized by a partial replacement of muscle potassium with sodium and a constant volume of muscle cell water despite discrepant changes in intracellular and extracellular total cation.

4. Cortisone ameliorated hypokalemic alkalosis despite the simultaneous administration of sodium salts. It is suggested that this effect may have resulted from the capacity of this hormone to produce tissue breakdown, thereby releasing potassium which is available to replenish the deficits of intact cells.

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THE PHYSIOLOGICAL BEHAVIOR OF RUBIDIUM AND CESIUM IN RELATION TO THAT OF POTASSIUM

Interest in the biological properties of rubidium and cesium stems from their close physicochemical relationship to potassium. Reference to the Periodic Table shows these elements to be adjacent members of the Group I alkali metal series, which has the order: lithium, sodium, potassium, rubidium, cesium. In terms of their chemical behavior the elements of this series may be separated into two groups, the division falling between sodium and potassium. Potassium, rubidium, and cesium display very similar electrical, chemical, and physical properties, which differ only quantitatively in a well-defined manner. When considered in this context, a comparison of the biological behavior of these ions can provide much important information to the investigator concerned with the mechanisms handling the alkali metals in the living organism. A study of the comparative biochemistry of rubidium and cesium is therefore an excellent, although indirect, method for investigation of the normal biochemistry of potassium.

The purpose of this paper is to review briefly certain aspects of the physiology of rubidium and cesium with particular reference to their relation to potassium. Space does not permit a complete or detailed discussion of this subject, and some active fields of investigation have been arbitrarily omitted. Emphasis has been chiefly placed on those topics most pertinent to mammalian, or at least vertebrate, physiology; newer developments in certain areas with which the author has had some personal experience have received greatest attention.

ANALYSIS OF K, Rb, AND Cs MIXTURES

The close chemical similarity of these ions gives rise to serious analytical problems. Flame spectrophotometry may be satisfactorily adapted to the analysis of certain mixtures of cesium and potassium,[61] but the close proximity of their spectral lines makes the analysis of mixtures of rubidium and potassium by this method a more complicated problem. Nevertheless, it has

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been reported that accurate determinations of rubidium in the presence of potassium have been carried out, using standard solutions containing appropriate concentrations of potassium.[9, 50] Most of the standard gravimetric or titrimetric methods capable of separating sodium from potassium do not separate potassium from rubidium and cesium. Thus, for example, precipitation as chloroplatinate, which will give accurate estimations of potassium in the presence of sodium, will not distinguish between potassium and its sister elements, rubidium and cesium.[87] Mixtures of these elements may be satisfactorily separated, however, by the use of paper chromatography,[73] or by ion exchange chromatography.[13, 14] Qualitative analyses of mixtures are probably most efficiently carried out by spectrographic techniques, but interfering effects of one ion on another make accurate quantitative analysis difficult.

When it is necessary only to measure rubidium or cesium in the presence of potassium, in a system not originally containing the former ions, labelling with radioisotopes offers a simple and satisfactory solution. Suitable isotopes of both elements are available. Rb^{86} has a half-life of 19 days, while the half-life of Cs^{134} is two to three years and that of Cs^{137} is 33 years; all three may be detected by their gamma radiation. In short-term experiments potassium may be analyzed simultaneously by K^{42} labelling, provided that the circumstances permit the attainment of a constant specific activity before cesium or rubidium is added to the system. The counting of samples containing K^{42} and one of the rubidium or cesium isotopes is accomplished by allowing the short-lived K^{42} (half-life 12 hours) to decay before the rubidium or cesium is counted. A satisfactory technique for experiments in which K^{42} cannot be used is to combine a chemical method with the radioisotope technique.[60] The total concentration of potassium and rubidium or potassium and cesium is measured by titration of chloroplatinate,[15] and the rubidium or cesium concentration is determined from the radioactivity. Potassium is then calculated by difference.

DISTRIBUTION IN THE ENVIRONMENT AND IN LIVING TISSUES

Of the Group I alkali metals, sodium and potassium are found in the earth's crust in the greatest abundance. The concentration of each is approximately 2.5 per cent by weight. The percentage of rubidium in the earth is roughly 1/2500th that of potassium or sodium, while the concentration of cesium is only 1/10th that of rubidium.[22] Spectrographic analysis has revealed rubidium to be present in virtually all animal tissue in the range of from 2×10^{-3} to 6×10^{-3} per cent (of dry weight),[7, 71] whereas tissue concentrations of potassium are usually some 300-800 times greater

than this. It is thus apparent that the abundance of rubidium relative to potassium is significantly greater in living tissues than in the terrestrial environment. Early spectrographic studies failed to identify cesium in animal tissues, but more recently this element has been found in the retinal tissue of various farm animals[67] and in the carcasses of a great variety of vertebrate species.[5] The average tissue concentration of cesium is reported to be about 3×10^{-3} per cent of dry weight.

The distribution in the body of naturally occurring rubidium resembles that of potassium, in that both elements are found in relatively high concentrations in red cells, muscle tissue, and viscera, with much less in bone and plasma.[6, 7, 71] No data are available on the distribution of cesium. However, tracer doses of radioactive rubidium[30, 40, 84] and cesium[27, 88] have been found to distribute themselves in the body with a pattern quite similar to that of the naturally occurring rubidium. All plant and animal cells studied so far have been found freely permeable to these ions. The permeability rates observed have varied considerably with the particular cell and the circumstance but are in general comparable to those for potassium.[10, 18, 20, 45, 65, 72, 79] When fed to animals in large quantity, rubidium is only slowly excreted in the urine[41, 54] and accumulates to a considerable degree in muscle and in most other soft tissues with a distribution similar to that of naturally occurring potassium or rubidium.[54, 55]

GENERAL PHYSIOLOGICAL EFFECTS

The physiological similarity between potassium and its chemical cousins, rubidium and cesium, was probably first noted by Ringer in 1882,[69] when he observed that the action of rubidium on the contractions of the isolated frog heart was almost identical with that of potassium. The effects of cesium were like those of potassium in some aspects, but the similarity between rubidium and potassium was much closer. In numerous investigations since then this general relationship has been demonstrated for a great variety of biochemical and physiological processes.[81] These include, for example, such diverse actions as the ability of these ions to neutralize the toxic action of lithium on fish larvae,[47] their effects on the motility of spermatazoa,[58] and the influence of varying extracellular ionic concentrations on the resting potentials in isolated nerve[21, 25, 32] and muscle[86] preparations and on the configuration of the electrocardiogram.[50, 75, 76] A similar relationship also exists with respect to the activating effects of the alkali metal ions on the release of acetylcholine by respiring brain tissue,[58] the utilization of Krebs cycle intermediates by isolated mitochondria,[57] the fermentation capacity of yeast,[43, 65] the active transport of sodium by frog skin,[34] and on the behavior

of isolated enzyme systems.[85, 92] Potassium and rubidium are often very nearly interchangeable, but cesium, although it resembles potassium more closely than sodium or lithium, is never a physiological substitute for potassium.

To the extent that rubidium or cesium are capable of substituting for potassium in biochemical and biophysical processes one would expect that these ions would be at least a temporary nutritional substitute for potassium. Thus, it has been shown that rubidium and, to a lesser extent, cesium can replace potassium as an essential nutrient for the growth of bacteria,[51, 52] yeast,[43] sea urchin eggs,[46] and rats.[34, 30] Young rats immediately cease growing on a potassium-free but otherwise adequate diet and usually die within a few weeks. The addition of rubidium to the diet will permit almost normal growth to occur for one or two weeks before the animals sicken again and die. To a more limited degree cesium is also capable of substituting for potassium in this way.[34, 55] Characteristic lesions develop in the kidneys and in the skeletal and cardiac muscles of potassium-depleted animals.[28] The addition of rubidium or cesium to the diet will prevent these changes[34] and if they have already developed, the feeding of these elements will rapidly effect a cure.[61]

ACID-BASE METABOLISM

Potassium depletion produced by diets low in potassium often results in marked changes in the acid-base balance of extracellular and intracellular fluid. There is evidence to suggest that loss of potassium from tissues is accompanied by migration of hydrogen ions from extracellular to the intracellular spaces, thereby increasing the acidity of tissues while producing an extracellular alkalosis.[18] The usual renal response to extracellular alkalosis would be expected to result in a diuresis of bicarbonate with subsequent restoration of plasma bicarbonate concentration to normal levels. With loss of intracellular potassium, however, the renal threshold for bicarbonate is increased as the result of accelerated exchange of hydrogen in tubular cells for sodium in the glomerular filtrate.[4] This increased threshold for bicarbonate prevents the excretion of the extracellular bicarbonate, and the urine remains relatively acid.

The administration of potassium to rats with diet-induced potassium-depletion and extracellular alkalosis corrects the acid-base disturbance chiefly at the tissue level by the restoration of normal tissue composition and the extrusion of hydrogen from cells back into the extracellular fluid.[18, 56] It has been shown recently that a similar displacement of intracellular hydrogen probably also occurs with the administration of rubidium

to normal or potassium-depleted animals, but the acidifying effects of rubidium can be even more pronounced than those of potassium.[59] The difference between potassium and rubidium is most marked in intact animals and least evident in nephrectomized animals. This suggests that the kidney is at least partly responsible for the acidosis.

Balance studies on normal intact rats loaded with rubidium or potassium support this suggestion for they demonstrate a significant reduction in ammonia excretion of the rubidium-loaded animals.[41] Simultaneously, these animals become much more acidotic than the controls which have been loaded with potassium. The cause of the suppression of ammonium excretion by rubidium remains to be elucidated. It is not apparently a function of urine pH because this was essentially unaffected in both groups; nor can it be attributed to any direct effect of rubidium on glutaminase activity, since *in vitro* studies with renal homogenates fail to demonstrate enzyme sensitivity to wide variations in rubidium or potassium concentration.[62] Tissue bicarbonate concentration is low in slices of rat kidney cortex depleted of potassium, and it is restored to normal by adding either potassium, rubidium, or cesium to the medium.[1] Consequently, there is no reason to believe that the ammonia effect is mediated through large differences in pH inside the renal tubular cells.

When administered to normal animals, cesium produces a milder degree of acidosis than does rubidium.[60] Like rubidium, however, it is capable of correcting the alkalosis associated with potassium depletion. No data are yet available on the effect of cesium on renal ammonia production.

RENAL EXCRETION

Considerable evidence supports the concept that the renal excretion of potassium is accomplished by tubular secretion of this ion in exchange for sodium in the filtrate.[8] There is also some reason to believe that tubular secretion of hydrogen occurs through a similar pathway and that, under some circumstances at least, tubular secretion of hydrogen and potassium are "competitive" processes.[4]

Initial studies on the renal clearance of rubidium carried out with tracer doses of Rb^{86} indicated that the clearance of rubidium was usually slightly less than that of potassium and tended to vary concordantly with the excretion of potassium under a variety of stimuli.[58] More recently, simultaneous potassium and rubidium clearances have been measured during the infusion of rubidium loads. In these experiments the rapid infusion of rubidium into normal dogs resulted in progressive elevation of plasma potassium and an increase in the excretion of potassium and rubidium. The excretion of

potassium during the administration of rubidium was slightly more rapid than during the administration of potassium loads which produced the same elevation of plasma potassium concentrations. Although the mean ratio of rubidium to potassium clearance was approximately 0.85, this ratio varied from .3 to 1.8. The ratio of rubidium to inulin clearance (C_{Rb}/C_{In}) varied from .2 to 2, without any definite relationship to plasma rubidium level. Demonstrable secretion of rubidium, arbitrarily defined as a C_{Rb}/C_{In} ratio greater than 1.10, occurred in at least 15 periods in four dogs.* It was always accompanied by still greater secretion of potassium and was observed following the administration of large doses of Diamox as well as occasionally during the slow infusion of rubidium into animals previously prepared by chronic loading of potassium. Further evidence for the tubular secretion of rubidium was afforded by an experiment in which the administration of BAL following the injection of a mercurial diuretic was observed to increase the excretion of rubidium as well as that of potassium. All these facts suggest that rubidium and potassium share the same renal transport mechanism. The differences between the rate of excretion of the two ions could be explained on the basis of a slower rate of secretion of rubidium by distal tubular cells.

Tracer doses of cesium in the dog are cleared at rates only about half that of potassium, and the excretion of cesium does not vary concordantly with the potassium excretion.[77] No studies on the excretion of cesium loads have yet appeared, and nothing is known of the transport mechanisms involved.

TISSUE ACCUMULATION

Earlier work had indicated clearly that animal tissues were capable of accumulating large amounts of rubidium or cesium.[50, 54, 55] More than half of the total tissue potassium content was replaced by rubidium when the latter was added to a low potassium diet.[50] It has recently been shown that most of the intracellular potassium of frog muscle can be replaced by rubidium or cesium if the tissue is allowed to soak in potassium-free Ringer solution to which rubidium or cesium has been added.[50] Experiments with tracer doses of radioactive rubidium[38, 49, 50] and cesium[38] in intact animals have shown that muscle tissue, red cells, and most viscera are capable of accumulating these ions against tissue-plasma concentration gradients equal to or greater than those existing for potassium.

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*Since there is no evidence of any binding of rubidium to dog plasma proteins,[40] the rate of filtration of rubidium can be estimated as the product of the plasma level and the inulin clearance.

In a series of experiments in which potassium-depleted, alkalotic young rats were fed equivalent mixtures of potassium and rubidium or potassium and cesium for periods of two to three weeks it was found that considerably higher concentration gradients were established for the rarer alkali metals than for potassium.[80] Up to two-thirds of intramuscular potassium was displaced by rubidium or cesium. Final intracellular/extracellular ratios for potassium remained normal and unaltered at approximately forty to one, but the ratio for rubidium was approximately three times this value while the ratio for cesium was roughly 4½ times the potassium value. It would thus appear that skeletal muscle of the rat accumulates these cations at equilibrium in a definite order of preference: cesium>rubidium>potassium.

The relationship described above between renal transport of rubidium and potassium could be explained in terms of this relative "preference" for rubidium in renal tubular cells. The rise in clearance of potassium produced by rubidium infusions could thus be due to displacement of potassium from secreting cells by rubidium. The relatively lower clearance of rubidium compared to potassium would then be a reflection of the relative tenacity with which rubidium maintains its intracellular position and resists exchange for sodium in the glomerular filtrate.

The observations on the relative uptake of the alkali metals have a critical bearing on some current concepts of ion transport in muscle cells. It was at first postulated that the accumulation of potassium was explained by a Gibbs-Donnan equilibrium resulting from the virtual impermeability of the normal muscle cell membrane to the influx of sodium and the efflux of the large intracellular anions.[8] When it subsequently became clear that the membrane was in fact quite permeable to sodium, the theory was modified to include the idea that sodium is actively extruded from muscle by an energy-requiring "pump," and that potassium accumulates as a result of the outward transport of sodium.[17, 19, 35, 74]

The potential difference generated by this continuous "sodium pump" is thought to provide an electrical field for the inward movement of potassium. Potassium is therefore conceived as moving into cells *against* a chemical gradient, but *down* the electrical gradient, the net result involving no change in electro-chemical potential and, hence, no active transport of this ion. Recent observations of ion transport by the isolated frog skin lend credence to this view, for they demonstrate that this preparation, at least, possesses an active transport system for sodium (and not for potassium) which is entirely responsible for the maintenance of a potential difference across the skin.[61]

If the above view were correct, one would expect similar concentration gradients at equilibrium for potassium, rubidium, and cesium, since they would be diffusing passively along the same electrical field. The large differences in gradient actually observed with these three ions therefore constitute a strong argument against this theory. Although it is conceivable that different mechanisms are involved in the transport of each of these ions, the existence of a single process is strongly suggested not only by the close chemical similarity of the ions but also by the fact that each ion of the group appears to competitively inhibit the penetration of the other two into frog muscle.[45] Thus, the simplest and most reasonable conclusion would seem to be that a single process is responsible for the cellular accumulation of all three and that this process cannot be simply passive diffusion.

If the "passive diffusion" theory is not valid for muscle, then at least two alternatives seem possible. The first is that in addition to the active outward transport of sodium there is also an active inward transport system for which these ions compete, the order of selectivity being Cs>Rb>K. This idea gains indirect support from recent studies with yeast,[17, 64] green algae,[66] and desheathed toad nerve fibers,[69] which indicate the existence of an active inward transport system for potassium which can operate without the simultaneous efflux of sodium. No direct support for the active and independent uptake of potassium by muscle has yet appeared, but several lines of evidence indicate that simultaneous movements of sodium and potassium are not always in stoichiometrical reciprocity, as might be expected if potassium accumulation depended upon a sodium pump.[17, 18, 70] Finally, there is evidence to suggest that potassium uptake by human erythrocytes is an active process, although it has not yet been demonstrated to be independent of sodium efflux.[28, 29] The kinetics of rubidium and cesium transport in red cells suggest that both these ions are handled by the same process responsible for active uptake of potassium, and they appear to compete with potassium for entrance into the cell.[18, 72]

A second alternative to the passive diffusion theory of potassium uptake is suggested by the analogy between the relative accumulation of the alkali metals by muscle tissue and the behavior of certain ion exchange resins, which selectively bind these ions in the same order of preference.[8] Selective binding of cations by fixed negative charges inside the cell has been proposed as a mechanism for the normal accumulation of intracellular potassium.[44] This hypothesis gains some support from the recent observation that relatively high concentrations of potassium are apparently maintained in frog muscle which has been soaked in potassium-free Ringer solution and poisoned with cyanide plus iodoacetate.[70] On the other hand, selective bind-

ing of cations within the cell would result in significant reductions in tissue osmotic activity as intracellular potassium is displaced by cations like rubidium or cesium for which the fixed negative charges have a higher affinity.[35] The absence of any evidence of water shifts in these experiments[36] therefore indicates either that large-scale binding does not occur, or that there exist cellular mechanisms which maintain total osmotic constancy despite fluctuations in cation activity.

At present there is no experimental basis for a choice among these or any other alternatives to the passive diffusion theory of potassium uptake. Obivously, much more information is required before an adequate description of ion transport in muscle cells can be given. It might safely be predicted, however, that similar or identical mechanisms are probably involved in the uptake of potassium, rubidium, and cesium. An explanation of the preferential accumulation of rubidium and cesium would for this reason contribute much toward the ultimate solution of the ion transport problem.

USE OF RUBIDIUM FOR DETERMINATION OF "POTASSIUM SPACE"

It has been suggested that because of its similar distribution in tissues, Rb^{86}, or small amounts of nonradioactive Rb^{85}, might be used instead of K^{42} in the determination of total exchangeable potassium or in the study of various aspects of potassium metabolism.[2,12] If this were so, use of Rb^{86} might offer considerable practical advantages over K^{42} because the former has a half-life of some 19 days, while the half-life of K^{42} is only 12½ hours. However, the observations discussed above indicate clearly that neither rubidium nor cesium can be used interchangeably with potassium for tracer studies, because tissues establish considerably higher concentration gradients for the two heavier elements than for potassium. One would expect that values for exchangeable potassium based on the ratios $\frac{Rb^{86}}{K^{39}}$ or $\frac{Cs^{137}}{K^{39}}$ in urine or blood would be spuriously high. A recent comparison of the "exchangeable" potassium calculated from the distribution of K^{42} and of Rb^{86} confirms this prediction, in that the Rb^{86} space was found to be significantly larger.[36]

TOXICITY

Although rubidium and cesium are capable of substituting for potassium in many biochemical processes, organisms will not tolerate indefinite replacement of potassium by either of these ions. Growing rats fed large quantities of rubidium or cesium, with or without the addition of potassium

to their diet, begin to show signs of toxicity within a few weeks, and eventually they all die.[24, 30, 61] No specific histological lesions are noted. The obvious manifestations of toxicity are chiefly in the neuromuscular sphere, with the animals usually becoming progressively more irritable and finally dying in convulsions. It is not yet clear how many of these symptoms are due to the acidosis usually produced by the administration of rubidium and cesium,[59] but preliminary experiments suggest that acidosis is not necessary to the development of toxicity.[60] Altered neuromuscular reactivity resulting from the profound changes in intracellular cation composition probably is an important factor, but more information is required before this question can be settled.

The acute toxic effects of rubidium infusions in the dog appear to be produced chiefly by the action of this ion on the electrical activity of the heart. Infusion of rubidium, which results in progressive elevation of plasma rubidium and potassium levels, produces a definite sequence of electrocardiographic changes which resemble in many ways the effects of hyperpotassemia alone.[29, 75] Thus, for example, when the plasma rubidium is 4 mEq./L and the potassium concentration is the same, one may observe peaking of T-waves which is quite comparable to the effect of elevating potassium concentration to 8 mEq./L. However, the presence of rubidium causes frequent ventricular extrasystoles and a reduction in amplitude of the S-wave, neither of which effects is seen regularly with infusions of potassium alone. Another difference between rubidium and potassium is revealed by the fact that dogs infused rapidly with rubidium die with ventricular fibrillation when the plasma concentrations of rubidium and potassium have each risen to levels of approximately 5 to 6 mEq./L, giving a total rubidium plus potassium concentration somewhat lower than the concentrations of potassium alone which produce fatal cardiotoxicity in animals infused with potassium.

These differences are probably explained by effects of rubidium on membrane potentials. It has been shown that the threshold potential of isolated myocardial fibers is decreased, and their spontaneous irritability usually augmented by a marked increase in potassium content of the bathing solution.[11] This effect is commonly assumed to be the result of a reduction in the concentration ratio $\left(\frac{K\ in}{K\ out}\right)$ term in the Nernst equation for the resting potential. The changes in the distribution of potassium caused by rapid infusion of rubidium might be expected to have a greater effect on myocardial irritability than would be predicted simply from the rise in serum potassium because the concentration of intracellular potassium is being

reduced simultaneously with the rise in extracellular concentration. The direct influence of the intra- and extracellular rubidium ions on the membrane potential is not known. If the tendency for rubidium ions to migrate out of cells was significantly less than that of potassium, replacement of some intracellular potassium by rubidium would tend to reduce the resting potential and thus perhaps contribute to the electrocardiographic changes observed.

SUMMARY AND CONCLUSIONS

This brief review of the physiology of rubidium and cesium has indicated some of the evidence for the close similarity of the biological effects of these ions to those of potassium. However, significant quantitative differences have been noted between the physiological behavior of these ions; cells do distinguish between them, and higher organisms will not indefinitely tolerate substitution of rubidium or cesium for potassium. Future attempts to explain the differences in the biological behavior of these ions in terms of their known physicochemical relationships should contribute much to an understanding of the biological systems involved. It may be suggested, for example, that the ultimate explanation of the mechanism underlying the preferential accumulation of rubidium and cesium by muscle tissue will probably reveal the nature of the process normally responsible for potassium uptake. The evidence available at present strongly suggests that this process is not a matter of simple diffusion.

The extent to which muscle tissues accumulate the "foreign" ions, rubidium and cesium, to the exclusion of potassium raises some interesting speculation. It seems clear that potassium owes its normal position as the chief intracellular cation solely to its relative abundance in the earth's crust. Were rubidium and cesium more abundant, there is every reason to believe that they, rather than potassium, would be the chief cations of muscle tissue. What consequences this would have had for the evolution of life is hard to imagine. In view of the toxicity of the heavier alkali ions it is apparent that the development of higher forms in an environment more rich in rubidium or cesium would have been contingent upon successful adaptation to the presence of these elements in the cell fluid.

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THE FATE OF INGESTED WATER

THE INTAKE OF WATER

Except in some of the lower orders, virtual constancy of the volume of body water is a prerequisite for the normal function and indeed the survival of the organism. In terrestrial animals, particularly, adaptive mechanisms provide for physiological regulation of the amounts of water which leave and enter the body. Although solid foods provide a considerable quantity of preformed water to the body, variation in water intake is largely dependent upon the volume of liquid imbibed. Physiological regulation of this volume is accomplished by the mechanisms of thirst and the drinking of water in quantities sufficient to satisfy thirst.

Referring to observations in animals, presumably less influenced than man by emotional and sociological factors, Adolph[3] has written, "Regulation of body content of water, therefore, is accomplished not merely by the overflow of any excesses. Equally, it is adjusted by measured intakes. Drinking is just as reproducible as excretion, and water balance is approached with equal accuracy from both sides." Wolf[198, 199] has presented equations and diagrams describing the quantitative relationships between water requirement and water intake in the dog and man.

Although thirst may be properly regarded as an entirely subjective phenomenon, it hardly seems pertinent to argue the validity of the inference that thirst exists in a dehydrated burro who, when offered water, promptly imbibes several gallons. It had long been recognized that "in man any large loss of fluid, as by sweating, diarrhoea, or haemorrhage, gives rise to an intense thirst that has its natural reaction in increased intake of water by mouth" (Starling[105]). However, Gilman's[46] observations demonstrated that a decrease in total body fluid was not required to stimulate drinking since dogs given hypertonic saline intravenously drank copiously even though body water was increased. A comparable rise in total solute concentration was produced by infusion of an equi-osmolar solution of urea, which unlike

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sodium chloride enters cells freely and therefore does not influence the movement of water across the cell membrane. Failure of the urea solution to evoke an equally large drinking response led to the view that cellular dehydration constitutes the stimulus for thirst. This concept has been generally accepted but it required some modification when Holmes and Cizek[82] and Cizek, Semple, Huang, and Gregersen[38] demonstrated that chronically salt-depleted dogs, whose body fluids were hypotonic and whose cells were presumably overhydrated, manifested an *increased* water intake. The fluid exchange of such animals returned to control values when the salt depletion was corrected with consequent expansion of their contracted extracellular and plasma volumes and elevation of body fluid tonicity to normal. The drinking responses of these salt-deficient animals, like the thirst evoked by isotonic losses of body fluid, cannot be ascribed to hypertonicity or cellular dehydration. Peters[84] has stated: "Thirst is not, therefore, preoccupied with the disposition or composition of the fluids of the body to the neglect of volume. The stimulus to drinking in behalf of volume appears to be the volume of the circulating blood which diminishes when the concentration of the sodium in the serum falls."*

It seems entirely reasonable to regard cellular dehydration and contraction of the extracellular fluid volume (or plasma volume) as the two most important physiological stimuli for thirst. The hypothesis that an osmoreceptor is involved in response to extracellular hypertonicity[128, 129] has been strongly supported by the work of Andersson.[8-9] In a brilliant series of experiments this investigator demonstrated the following:

1. Injection of very small amounts of hypertonic saline directly into a hypothalamic area located medially in the vicinity of the third ventricle evokes an immediate and striking drinking response in goats. As much as eight liters of water may be drunk after a single hypertonic injection. Injection of isotonic saline does not produce such an effect.

2. Electrical stimulation of the same area also causes marked polydipsia.

3. In dogs destructive lesions involving the area concerned produce a marked decrease in water intake, without impairment of food intake. Marked dehydration develops in such animals.

The last observation suggests that integrity of the hypothalamic "thirst center" is required for appropriate drinking responses to be evoked by contraction of volume as well as by increase in tonicity, since both stimuli must have been present when dehydration ensued. It is of course possible that a separate "volume receptor" is located in this same area.

* This quotation is from the manuscript of the revision of *Quantitative clinical chemistry*, Vol. II, *Interpretations*.

The foregoing discussion of thirst in terms of osmotic and volumetric stimuli has omitted a number of relevant observations. Holmes and Montgomery[97, 98] studied the responses of blood donors and dogs bled up to 30 per cent of their blood volume. In the absence of circulatory shock thirst was not evoked. However, shocked dogs drank avidly.

Other studies indicate that not only cellular dehydration, but also total solute concentration influences water intake. In rats the administration of hypertonic urea solutions by stomach tube evoked a marked drinking response beginning in 15 minutes.[4] In Gilman's[48] experiments urea in hypertonic solution did evoke drinking of about half the amount taken after an iso-osmotic sodium chloride load; such comparisons were greatly extended by Holmes and Gregersen.[65, 66] Platt[95] has described his own thirst following urea ingestion, but does not indicate whether diuresis occurred before thirst ensued.

It should also be emphasized that reduction of salivary flow regularly accompanies thirst induced either by water loss or the administration of hypertonic saline.[60, 64] Moreover, sham drinking in esophagostomized dogs produces temporary inhibition of drinking responses,[1, 11, 113] as does distention of the stomach with fluid or balloons in various species.[3, 4, 11, 65, 88, 113] Such observations have led Montgomery and Holmes[88] to propose a multiple control system for metering the volume of fluid taken in response to a given thirst-producing stimulus:

1. Passage of water through the pharynx and esophagus gives satisfaction for 20 to 30 minutes.

2. Gastric distention becomes an effective inhibitor of additional drinking for the next hour.

3. During this hour absorption of the ingested water occurs with correction of hypertonicity and cellular dehydration and removal of the thirst-producing stimulus. (The time relations given apply to the dog.)

A variety of other determinants also modify drinking responses,[2, 4] including emotional and sociological factors at least in man. The proposed control system serves to integrate a variety of individual reflexes into an organized regulatory mechanism.

WATER IN THE ALIMENTARY TRACT

Ingested fluid represents an exogenous addition to the intrinsic secretions of the gastrointestinal tract. Even when tap water, virtually devoid of solute, is taken by mouth in large quantity, the ingested fluid tends to become isotonic. It acquires electrolyte by admixture with saliva, gastric juice, and secretions present in the intestinal lumen. The volume and com-

position of the gastrointestinal secretions has been described in detail elsewhere.[29, 43, 44, 45, 88, 92, 98] The copious secretions produced each day are of course reabsorbed almost completely. No technique has been devised to measure in the living organism the volume of fluid present in the gastrointestinal tract at any given time. Everyday clinical experience indicates that the stomach of man in the post-absorptive state normally contains at most 100 ml. of fluid.[57] The volume that can be aspirated is slightly smaller than the so-called "pooling space"[99] and does not include other "transcellular" fluid[98] such as that in the mucosal glands. It is well established that water in the gastrointestinal tract constitutes a very considerable fraction of the total body fluid in certain species. In rabbits[22, 49] and guinea pigs,[22] 12 to 20 per cent of the total body water is normally present in the gastrointestinal tract. In fed rats[98] and dogs[98] about 7 per cent is present, a value which falls below 5 per cent when food is withheld.

A group of observations by Edelman and his colleagues[38, 40, 80, 110] show that the intestinal tract of human subjects examined at post-mortem contains a far smaller moiety of the body water. Direct measurement of fluid in 12 post-mortem examinations gave an average value for total gastrointestinal water of 1.4 per cent of predicted total body water, of which 0.4 per cent was in the stomach, 0.7 per cent in the small bowel, and 0.3 per cent in the colon. Average values for gastrointestinal sodium, potassium, and chloride ranged between 1 and 2 per cent of the predicted total exchangeable amounts. The authors properly emphasize the limitation imposed upon interpretation of these post-mortem data by possible changes in intestinal fluids occurring during terminal illness and in the hours after death.

It is clearly impossible to estimate the extent to which the composition of a given volume of tap water taken orally will be altered immediately by mixing with saliva, gastric, and intestinal contents. That some water may enter the duodenum virtually unaltered in composition is suggested by Ivy's[74] observation that water begins to leave the accessory stomach of Pavlov pouch dogs within a few seconds after it is introduced, the emptying time for 400 ml. being 30 to 60 minutes. Since man may deliver 400 ml. of water from stomach to duodenum in 15 minutes after it is drunk,[74] the inference is strong that this transport begins immediately after drinking, as in the dog. Moreover, it is well known that an aqueous suspension of barium sulfate may begin to pass through the pylorus almost as soon as it is swallowed. However, the available studies[42, 76, 96] indicate that after the administration of moderate amounts of water the duodenal and jejunal contents are soon restored to approximate isotonicity. Conversely, hypertonic solutions introduced into the alimentary tract decrease in total solute con-

centration. Isotonic saline instilled through a Miller-Abbott tube is altered in chemical composition, so that without change in total cation concentration, chloride is replaced by bicarbonate, the extent of such replacement being greater as the fluid moves in an aboral direction.[18] This observation concerning change in ionic pattern is consistent with many other studies in various species.[18, 27, 28, 121] Thus fluid added to the gastrointestinal contents tends not only to become approximately isotonic but also to acquire the ionic composition of the endogenous secretions present in the area.

Although there are conflicting reports concerning the presence of precise osmotic equality between gastric juice and plasma,[44, 47, 81, 111] there is no doubt that the tonicity of the gastric secretions varies directly with that of the plasma. A primary change in the total solute concentration of serum evokes a comparable change in the osmolarity of the gastric juice.[47, 111] Pancreatic juice[10, 45, 75] and hepatic duct bile[45, 96] are approximately isotonic. The jejunal secretions are isotonic and have been shown to be in osmotic equilibrium with the plasma.[66, 96]

The tendency of exogenous fluid to be rendered isotonic in the gastrointestinal lumen doubtless prevents the occurrence of significant hemolysis when plain water is drunk. That fluid need not approach isotonicity prior to absorption or during the process of absorption is most clearly established by the fact that hemolysis can be produced in portal vein blood of rats, cats, and rabbits by instilling large water loads directly into the duodenum.[90] Such hemolysis apparently takes place in the first few minutes during which absorption is thought to be rapid because of increase of intraluminal pressure[16, 90, 123, 124] and there has been little time for electrolyte to enter the instilled water. Hemolysis in portal blood would also be prevented by absorption of water via lymphatic pathways but this does not occur to a significant degree, at least in the rat.[12, 77] Important protection against hemolysis when water is taken by mouth is no doubt provided by the entrance of electrolyte into the water taken during its transit through the stomach.[5] Hunt has examined the possibility that gastric emptying is delayed when instilled solutions are hypotonic or hypertonic, permitting a change toward isotonicity before the fluid enters the small intestine.[69, 70] Examination of the responses to a variety of solutions led to the conclusion that although an osmoreceptor mechanism does indeed influence gastric emptying, its operation apparently does not serve to render isotonic the solutions delivered to the duodenum. Finally, it is possible that insofar as the intestinal mucosa may come into contact with fluid which is markedly hypotonic, injury to the epithelium is likely to occur[15, 80, 112] with delay in absorption and consequent lessening of the likelihood of hemolysis.

ABSORPTION OF WATER BY THE INTESTINE

The foregoing attempt to indicate the nature of change which occurs in the composition of ingested fluid during its sojourn in the gastrointestinal tract has dealt only with plain water and solutions of sodium chloride. Consideration of other solutions or foods will not be undertaken. It should be emphasized that change in the volume and composition of ingested fluids is not merely the result of admixture of intestinal content already present or of secretion added during gastrointestinal transit but also of absorption by the intestinal epithelium. Indeed, it is this very phenomenon of simultaneous movement of water and various solutes across the intestinal wall in both directions at different rates that has made the hypotheses concerning intestinal absorption inordinately complicated and contradictory. If the recent applications of isotopic tracer studies have not simplified the problem, they have at least provided answers to certain controversial matters of fact. For example, it had been accepted for many years that net absorption of water in the stomach is negligible.[108] However, tracer studies indicate clearly that D_2O rapidly enters the blood stream from the stomach and equilibrates with the water of the body.[34, 39, 105] Since the outflux of water is normally balanced by an equal or greater influx, no net transfer out takes place.

The historic controversy between those who argued that movement of water and electrolyte across the intestinal wall could be accounted for by osmotic and hydrostatic forces alone and those whose observations or interpretations required a "vital force" (Starling[108]) to explain such movement has been reviewed at intervals.[48, 60, 100, 117] Contemporary studies employing tracer techniques indicate that active transport dependent upon metabolic processes of the cells is clearly involved even though the direction and magnitude of such transport is regulated, at least in part, by osmotic relationships. The proponents of the adequacy of physical forces provided a great deal of information concerning the manner in which osmotic and hydrostatic pressures modify the processes of intestinal absorption. It has been repeatedly demonstrated that water is more rapidly absorbed from hypotonic than from isotonic sodium chloride solutions.[100, 101, 117] It is even more slowly absorbed from hypertonic sodium chloride solutions and there is no net absorption from isotonic solutions of nonabsorbable solutes, such as $MgSO_4$. Osmotic forces clearly influence movement of water across the intestinal epithelium. The possible actions of the osmotic pressure of colloids in intestinal lymph and the intraluminal hydrostatic pressure to promote water absorption have been emphasized by Wells.[123, 124] His suggestion that the protein-rich fluid of the lymphatics provided the high oncotic pressure to effect the movement of water out of the intestinal lumen was not

considered incompatible with ultimate transport via the portal venous system.[94, 118] However, subsequent studies have shown that when D_2O is ingested its specific activity rises markedly in portal vein blood but not in the mesenteric lymphatics of the rat.[12] The specific activity in lymph remains identical with that of arterial plasma. These observations would seem to exclude entrance of the absorbate into the lymph of the villi at any step in the process, a most serious objection to Wells' concept. On the other hand, it is generally accepted that increased intraluminal pressure may favor transfer of water out of the lumen.[16, 90] Of course, at high intestinal pressures important circulatory disturbances take place which interfere with absorption.[20, 88, 89]

The most extensive studies concerning intestinal absorption during the past twenty years are those of Visscher and his group who in 1938 proposed the fluid circuit theory.[71, 73, 91] This schema hypothesized a continuing circuit in which fluid leaves the intestinal lumen to enter the blood stream by passing through a membrane permeable to univalent ions such as Na^+ and Cl^- but impermeable to polyvalent ions such as Mg^{++} and $SO_4^=$. Fluid is returned to the lumen from the plasma through a membrane relatively impermeable to all ions. It was further postulated[73] that anomalous osmosis[54, 55, 82] provides the necessary pumping action to maintain the fluid circuit. The behavior of isotonic mixtures of NaCl and Na_2SO_4 or of NaCl and $MgCl_2$ instilled into the small intestine is consistent with Visscher's hypothesis. The former mixture becomes virtually chloride free, leaving an isotonic solution of $NaSO_4$;[21, 73] the latter mixture becomes markedly depleted of sodium leaving an approximately isotonic mixture of magnesium chloride and bicarbonate.[73] In each case one univalent ion is almost completely removed from the intestinal fluid. The univalent ion species which remains in the ileal fluid is restrained by the nondiffusible polyvalent ion of opposite charge. The impermeability of the membrane which restrains the return of univalent ions to the lumen was thought to be more complete for Cl^- than for Na^+, since Cl^- is more completely removed from the lumen than is Na^+. The virtually complete impermeability for Cl^- was also considered to be linked with the appearance of bicarbonate, producing a Cl^-—HCO_3^- exchange. However, the independent secretion of $NaHCO_3$ by intestinal glands would produce the same result.

Subsequent observations demonstrated unequal bidirectional fluxes of sodium and chloride, as well as of water, across the intestinal epithelium,[119, 122] requiring modification of the concept of one-way permeability for these ions. It was further shown that the net absorbate from an isotonic solution instilled into ileum is hypertonic,[81, 120] and that net transfers of water from solutions of various tonicities are far greater than predicted by osmotic

theory.[119] Osmotic forces were shown to influence the direction and amount of net transfer but could not account for the magnitude of the exchanges. The suggestion was made that the rôle of intraluminal osmolarity is to alter the water content of the surface epithelial cells which might in turn influence their activity in the transport of fluid.[121]

In discussing the observations of Visscher and his co-workers[119] concerning rates of water movement which differ from those predicted by osmotic theory Ussing[115] states, "There can be little doubt that active processes do play a rôle. . . . It seems, however, that the osmotic pressure of the gut contents is of greater importance than the active transport, judging from the fact that water enters the gut in excess when hypertonic solutions are applied. As we have seen above, the deviation from ideal behavior in itself does not, unambiguously, indicate active transport." Additional discussion is given elsewhere.[114, 118]

Although the fluid circuit schema in its original form has not been emphasized by Visscher in recent publications,[58, 118] the model remains useful as a dynamic description of the movement of fluid across the intestinal mucosa. The rates of influx and outflux of water and ions are presumably in part anatomically determined; thus the ileum is characterized by a relatively rapid outflux and slow influx of univalent ions. The tonicity of the intraluminal fluid exerts an all-important influence upon these rates of flux, at least the outflux of water and univalent ions. In this way the magnitude and direction of net transfer of water is controlled by the effective osmolarity of the intestinal fluid even if the energy for active transport must derive from the metabolic processes of the cells.

THE DISTRIBUTION OF ABSORBED WATER

The transport of ingested water across the intestinal barrier has long been assumed to take place quite rapidly. This assumption is an entirely reasonable inference based upon the remarkable consequence of the imbibition of a large amount of water, namely, the prompt excretion of an approximately equal quantity of watery urine. The phenomenon of water diuresis is discussed in another contribution[135] to this memorial issue. Some consideration will be undertaken here of the fate within the body of such a large oral intake of water.

The assumption of free permeability of virtually all cell membranes of the body to water leads to the prediction that the ingested water when absorbed would be uniformly distributed throughout the several compartments of body water if retained long enough to permit equilibration to occur. At equilibrium, the aggregate concentration of osmotically active solute would

be lowered equally in plasma, interstitial fluid, and cell water. The percentage fall in concentration would be equal to the percentage rise in volume of total body water. Moreover, the absorbed water would be allocated to the several compartments in proportion to their respective volumes. Thus if a 70-kilogram adult man drank and retained two liters of water, equivalent to 5 per cent of the total body water, at equilibrium there would be anticipated a 5 per cent fall in the total solute concentration and in the sodium and chloride concentrations as well. Moreover, if the plasma volume were expanded proportionately, a 5 per cent fall in serum protein concentration and a roughly equal fall in hemoglobin concentration would be observed.*

The classical experiments of Haldane and Priestley[38] in 1916 indicated, however, that little change in hemoglobin concentration took place when a large volume of water was taken orally. On the other hand, a distinct fall in conductivity of the serum, a measure of electrolyte concentration, was observed.[38] Although at this time no basis existed for predicting the magnitude of decrease in these values to be anticipated with a water load of given size, the unequal change in the two measures of hemodilution was most perplexing. The paradox was plausibly resolved by Priestley's[38] ingenious suggestion that the salts of the blood migrate into the gastrointestinal tract. However, in a subsequent paper Priestley[37] implied that the ingested water initially enters the blood stream and subsequently diffuses out into the tissues and alimentary canal, abstracting salts from the blood in the process. Moreover, the additional data he obtained indicate that although the fall of about 5 per cent in blood chloride observed after imbibing two liters of water is of a magnitude comparable to the previously reported decline in conductivity, there is actually a significant fall in hemoglobin and total solids as well, amounting to about 2 to 3 per cent in most experiments.

A number of investigators pursued this problem during the next twenty years. The variety of measurements employed to indicate the distribution of the ingested fluid included serum conductivity, concentration of chloride, bicarbonate, sodium and potassium, serum and whole blood total solids, as well as hemoglobin and serum protein concentrations. Comparison and generalizations are rendered exceedingly difficult, even if only studies in man are considered, owing to the diverse parameters employed as well as variation in the magnitude of water loads and the times of sampling. An extensive citation of the literature prior to 1937 is given by Findley and White.[41] The majority of the observations point to a demonstrable hemo-

* Given a 5 per cent increase in plasma water and red cell water the hemoglobin concentration per 100 ml. of whole blood *water* would decrease by exactly five per cent.

dilution as reflected by change in total solids, hemoglobin, or serum protein concentration, when water loads of more than a liter are taken by normal man. With much larger loads given to experimental animals, as in the studies of Greene and Rowntree[61] concerning water intoxication in dogs, very large changes in hemoglobin and serum protein concentrations were encountered. In the majority of studies the observed fall in serum electrolyte concentration is larger than the decline in blood colloids, suggesting diffusion of the crystalloids out of the vascular bed presumably into the water as yet unabsorbed from the intestinal tract.

The possibility that vasomotor changes might account for some of the inconsistencies in data pertaining to nondiffusible constituents of venous blood has been considered. The data of Govaerts and Cambier[90] obtained on arterial samples in ten women are usually consistent in showing an average fall of approximately 1.0 per cent in serum total solids, 1.4 per cent in conductivity and 1.7 per cent in serum chloride 30 to 60 minutes after the subject drank a liter of water. On the other hand, they reported very variable changes in hemoglobin.

The known effect of large oral water loads to reduce body temperature, even when the water taken is warmed,[62] supports the suspicion that the composition of peripheral venous blood after water drinking may be modified by changes in cutaneous blood flow. This has led to the employment of arterial or "arterialized"[97] samples in a number of contemporary studies.* After its oral administration the concentration of D_2O in peripheral venous blood exhibits fluctuations not observed in arterial samples.[24]

Moreover, attempts have been made to elucidate changes related to alimentary water exchange by comparing oral and parenteral routes of water administration. The most extensive study is that of Hollander and Williams[61] who compared the oral administration of tap water with the intravenous administration of a 4 per cent solution of invert sugar in a group of essentially healthy adult male subjects. The two techniques of water loading produced essentially the same decrease in the concentration of osmotically active solute. However, calculated changes in plasma volume indicated an actual reduction during oral loading in many of the studies, whereas intravenous administration was almost uniformly associated with an apparent expansion of intravascular volume. It should be noted that

* The writer is unaware of any systematic comparison of changes observed in conventional venous blood samples with those found in arterial blood after water loading in normal subjects. In a few studies in patients with hepatic cirrhosis, Birchard[18] found no consistent difference between venous and arterial changes, nor were the changes in arterial blood more uniform or predictable.

until the parenterally administered hexose enters cells it provides an effective osmotic force retaining water in the extracellular space. Hence the distribution of the administered water may be expected to differ from the uniform partition among the several compartments of body water to be expected if plain water were given. The plain water taken by mouth would produce a much smaller expansion of plasma and interstitial volumes if uniformly equilibrated through the body fluid compartment, but such equilibration could hardly produce a *contraction* of plasma volume. The authors infer, as did Welt and Nelson,[120] that the water imbibed continuously throughout the experimental period maintains a significant pool of solute-poor fluid in the gut lumen into which extracellular electrolyte migrates to produce hypotonicity of the interstitial fluid. Fluid segregated in the gut obviously cannot expand the volume of plasma or that of any other body fluid compartment. However, the induced hypotonicity of interstitial fluid produces an osmotic gradient for movement of extracellular water into cells, with reduction in interstitial and plasma volumes.

It must be emphasized that direct evidence for intestinal segregation of water in these experiments is lacking. Dicker[88] studied responses to water loading in rats with nutritional hypoproteinemia. The fall in serum chloride was similar to that observed in normal animals after water loading, but the increase in plasma volume observed in the normal animals was lacking. The inference of segregation in the gut would have been quite plausible if direct observation had not demonstrated that the protein-deficient animals absorbed water even more rapidly than did the normals. The area of pooling in these animals appeared to be in the connective tissues, particularly retroperitoneally. The possibility that patients with hepatic cirrhosis rapidly segregate ingested fluid in areas other than the intestinal lumen has been considered by Birchard and co-workers.[14]

It is obvious that equilibration of administered water cannot take place as long as a continuous addition is being made either orally or parenterally. If a water load is maintained by drinking a volume calculated to replace losses during water diuresis, the magnitude of the gastrointestinal pool of fluid will depend upon the net rate of absorption and also upon the rate of diuresis which determines the prescribed intake. Since the maximal rate of water diuresis tends to fall in seated subjects[104] and since water absorption may be less rapid in recumbency,[107] the size of the intestinal pool may be significantly influenced by posture. Nor is equilibration achieved by the normal organism when a single large water load is taken. The studies of Smirk,[108] who employed an ingenious technique of differential weighing of abdomen and legs, clearly demonstrated that normal men absorbed a liter

of ingested water in 25 to 55 minutes. This absorption is characteristically followed by a marked rise in renal excretion of water within half an hour. Thus, the diuresis is likely to begin before complete equilibration with the body fluids occurs.[84, 85]

Attempts to achieve equilibration have therefore been carried out when renal excretion of water has been eliminated surgically or minimized by pitressin administration. Other studies designed to elucidate the osmotic behavior of the cells have been made following the administration of solute loads. No final agreement has as yet been achieved concerning several most fundamental aspects of the distribution of body water.

It has been claimed that the osmotic pressure within cells exceeds that of the extracellular fluids and that water is continually being pumped out of the hypertonic cells by an active process. The literature concerning this question has been reviewed by Robinson.[102, 103] The interpretation of Conway and McCormack[25] that their cryoscopic measurements of various tissues demonstrate isotonicity of cells and surrounding fluid has been questioned by Brodsky and co-workers.[17] On the other hand, Leaf[78] has shown that water taken up by surviving tissues *in vitro* is accompanied by solute in approximately isotonic proportions; hence, swelling of cells under these circumstances cannot be taken as evidence that the intracellular fluids are normally hypertonic with respect to their extracellular environment.

Demonstration that intracellular and extracellular osmotic pressures are equal would constitute strong evidence that the cells behave as osmometers. The converse that if they do behave as osmometers, osmotic equality must pertain does not follow. As stated by McDowell, Wolf, and Steer,[84] "It may be true or not that osmotic pressure within cells exceeds that outside of cells; but neither case is necessarily incompatible with the finding that cells act volumetrically as if they were osmometers when osmotic stresses are applied."

The distribution of a large administered water load constitutes one test of the osmometric behavior of the cells. Detailed analysis of data which indicate transfers of water which deviate from predictions based on electrolyte balance have led to the hypothesis that the cellular constituents exhibit variations in their osmotic activity which modify the distribution of body water.[37–40] However, in two recent studies[79, 121] involving anuric dogs and one oliguric human subject, large administered water loads induced changes in plasma composition which were in accord with free permeability of the cells to water and constancy of the total cellular osmotic activity.

The majority of the studies which led to the hypothesis of change in the osmotic activity of cell constituents involved large external transfers of

electrolyte as well as of water. The recent studies of McDowell, Wolf, and Steer[64] may therefore be more pertinent to this general problem. Their studies indicate that in response to administration of hypertonic solutions of sodium salts and of sucrose, the osmolarity of the body fluids rises more than can be accounted for by the exogenous increment of solute alone. The "idiogenic" changes in osmotic pressure did not occur when urea loads were given, leading to the inference that cellular dehydration might evoke the formation of additional osmotically active particles. The possible relationship of such osmotic changes to muscular activity[197] and to anoxia[68] is recognized by the authors.

It appears therefore that even under rigorous experimental conditions designed to permit the attainment of equilibrium, the precise distribution of water within the body may not be predictable.[180] However, apparently anomalous behavior probably represents incomplete assessment of the osmotic forces operative rather than encroachment upon the freedom of water to move in response to such forces.

CONCLUDING REMARKS

The selection of topics for this discussion has been quite arbitrary; the treatment largely descriptive. No attempt has been made to choose material illustrative of a particular concept or principle. Despite the scattered array of isolated observation and general statement, there is ever apparent the dual aspect of the physiology of body water: regulation of volume and regulation of tonicity. If thirst is evoked by hypertonicity, water drinking is metered by a control system responsive to the volume of fluid in the stomach. The rate of water absorption in the intestine varies inversely with the tonicity of the solution presented to the mucous membrane. The volume of water normally remaining is very small, whether the fluid intake is less than a liter or several gallons and whether the concentration of absorbable solute is quite high or very low. Understanding of the immediate fate of ingested water is seriously limited because even though lowering of solute concentration in body fluids is a regular event, the changes in plasma and extracellular volume are variable and not susceptible of precise measurement. The volume of cells has been regarded as a function of the tonicity of extracellular fluid. The rôle of primary changes in intracellular osmotic activity in the regulation of cell volume requires further investigation. Certainly the provocative suggestion that the volume of the cells is determined by active transport of water rather than regulated by osmotic forces has already stimulated a search for new knowledge concerning these relationships within the cells themselves.

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RENAL EXCRETION OF SODIUM AND THE CONCEPT OF A VOLUME RECEPTOR‡

The mechanisms by which a healthy individual maintains constant the volume of his plasma and extracellular fluid have interested and baffled physiologists for years. The problem is peculiar to multicellular, complex organisms; our unicellular ancestors, enclosed by a semipermeable membrane and swimming in a sea of constant composition, had only to maintain intact the effective osmotic concentration inside their cells for cellular volume to adjust itself automatically. When they became larger and moved on land, taking with them a bit of the primeval ocean in their extracellular fluid, it became important to preserve not only the composition, but also the volume of the fluids bathing their cells. Bodily regulations which serve these ends must necessarily be concerned with the intake and excretion of the chief inorganic constituent of extracellular fluid, namely, the sodium ion. It is for this reason that studies of the regulation of sodium excretion by the kidneys have attracted physiologists and clinicians primarily interested in such diverse states as dehydration, congestive heart failure, nutritional edema, anemia, hemorrhage, and hypertension.

It was recognized clearly by Ernest Starling,[57] though not by some later physiologists, that the balance of forces across the capillary membrane which he described explains only the localization of salt and water in edematous tissues, not their over-all retention by the body. It is tautologous to say that the kidney normally operates in such a way that the volume of extracellular fluid is maintained constant; to delineate clearly the mechanisms by which this is accomplished, it is necessary not only to describe the intrarenal factors affecting the excretion of sodium, but to examine the bodily processes through which the kidney is notified to retain or excrete this ion. Hence the search for a "volume receptor"—an afferent mechanism sensitive to some crucial function of the volume of body fluids.

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RENAL MECHANISMS FOR SODIUM EXCRETION

Of primary importance in any attack on the problem of the regulation of the volume of body fluids are the circulatory, hormonal, and neural factors directly influencing renal excretion of sodium.[8] All other things being equal, even small changes, immeasurable by present techniques, in the rate of glomerular filtration inescapably alter the rate of sodium excretion. Because glomerular filtration rate and renal blood flow so frequently are found to change in the same direction as sodium excretion in a variety of clinical and experimental situations, it has correctly been concluded that they are powerful determinants of the excretion or retention of sodium, but it is too often assumed that they are the only, or even the most important, determinants. Here physiologists specializing in the tractable dog are at some disadvantage when attempting to extrapolate to the human. Glomerular filtration rate in the dog is much more readily changed than in humans. The dog initially excretes a load of salt largely by increasing the rate at which glomerular fluid is filtered.[26] Intravenous saline loads in man, on the other hand, may produce no measurable increase in glomerular filtration rate, even when they provoke a huge sodium diuresis.[11] Renal retention of sodium in man during quiet standing can be demonstrated to occur even when a fall in the amount of filtered sodium is prevented by simultaneously infusing albumin or hypertonic saline.[17] The administration of digitalis to cardiacs in congestive failure is followed by a diuresis of sodium and water before any change is apparent in renal hemodynamics.[23] Cirrhotics with normal renal clearances of inulin and para-aminohippurate retain sodium with avidity even when it is given in hypertonic solution.[29] Opening an arteriovenous fistula is followed by a drop in sodium excretion, while closing it provokes an increase, all without measurable changes in renal blood flow or glomerular filtration rate.[19] Even the separately perfused kidney can apparently vary its excretion of sodium widely without altering its blood flow or filtration rate. Selkurt has shown that increasing or decreasing renal arterial perfusion pressure produces an increase or decrease in the excretion of sodium and water without parallel changes in the formation of glomerular fluid[28]; this suggests that intrarenal pressure changes may be reflected in alterations in tubular behavior.

The possibility that changes in the intrarenal distribution of blood among nonhomogeneous nephrons may alter sodium excretion has been recently reopened by the experiments of Pappenheimer which suggest that cortical nephrons are perfused by blood having a different hematocrit from nephrons in the medulla.[46] If certain nephrons handle a disproportionate share of glomerular filtrate in a different way from their fellows, it would not be

hard to visualize readjustments occasioned by changes in renal arterial perfusion pressure which might, for example, result in a greater proportion of fluid being filtered through the glomeruli of long, sodium-retaining nephrons and a smaller proportion through short nephrons unable to reabsorb as much sodium. This kind of readjustment would not be detected by the usual (Fick) clearance techniques which measure only the total flow of blood to an organ. If indeed there is considerable nonhomogeneity of blood flow to nephrons within the kidney, traditional concepts of the division of responsibility for sodium retention between alterations in "glomerular filtration" and active changes in "tubular reabsorption" in a variety of states may have to be revised. Subject to the above considerations it appears that in man hormonal and possibly nervous influences on tubular reabsorption of sodium play a major rôle, in addition to changes in glomerular filtration rate, in determining whether sodium is to be retained or excreted.

NEURAL INFLUENCES ON SODIUM EXCRETION

The regulatory rôle of the autonomic nerves to the kidney has been assigned variously to the renal vasculature alone[59] or to the control of tubular function, including the active reabsorption of sodium in the proximal tubules as well.[60] Because of the relatively inexact techniques available for measuring small changes in tubular handling of a substance so largely reabsorbed from glomerular fluid as sodium, it has been difficult to amass data proving that renal tubules are in fact responsive to renal denervation or to stimulation of the renal nerves, apart from the changes in renal hemodynamics which frequently accompany such maneuvers. Renal denervation in an animal under anesthesia usually results in an increase in sodium excretion, glomerular filtration rate and blood flow in the denervated kidney, while experiments in unanesthetized dogs have generally been unsuccessful in detecting differences between denervated and innervated kidneys.[58] It seems reasonable to conclude that "denervation diuresis" may be a consequence of the release of enhanced vasoconstrictor activity under anesthesia, especially since small changes in glomerular filtration occurring acutely are known to produce large percentual changes in sodium excretion. Sympathectomized patients, in whom nervous connections to the kidneys have presumably been interrupted, usually retain or excrete sodium in normal fashion so that plasma and extracellular fluid volumes are maintained intact; furthermore, they develop sodium retention and edema when congestive heart failure supervenes. Moreover, as will be seen below, they promptly retain sodium when blood is acutely sequestered in the limbs by congesting cuffs even though glomeru-

lar filtration and renal blood flow are not measurably depressed by this maneuver. Complete renal denervation fails to prevent the fall in glomerular filtration rate and sodium excretion caused by primary reduction of the cardiac output[9] and high spinal anesthesia does not restore the diminished renal blood flow and filtration rate or increase the rate of urine excretion in patients with congestive heart failure.[45] Autonomic blockade with hexamethonium or unilateral renal denervation does not prevent the reduced excretion of water, sodium, and chloride which is induced by hemorrhage, even though the arterial blood pressure is well maintained and there is no significant change in filtered sodium and renal plasma flow.[37] It would appear that renal innervation is not essential in men or dogs to the decreased excretion of sodium which follows reduction in "effective blood volume."

HORMONAL INFLUENCES ON SODIUM EXCRETION

There is considerable evidence that in almost every clinical state characterized by the formation of edema the renal tubules are influenced by salt-retaining hormones manufactured by the adrenal cortex and secreted in supernormal amounts. Increased quantities of aldosterone have been detected in the urine of patients with congestive heart failure,[1] cirrhosis with ascites,[1] toxemia of pregnancy,[61] and nephrosis.[43] In patients with these disorders the output of aldosterone appears to fluctuate in parallel fashion with the intensity of the circulatory stimulus to salt retention.[41] For example, patients with cirrhosis and ascites demonstrate a greatly increased excretion of aldosterone immediately after paracentesis, when the rate of transudation of fluid from the blood stream is greatest.[1] In addition, blood loss,[38] sodium restriction,[40] and dehydration of the *extracellular*[3] fluid call forth an increased output of sodium-retaining hormone. On the other hand, selective dehydration of the *intracellular* fluid compartment induced by the infusion of hypertonic saline is not accompanied by an increased titer of salt-retaining substances in the urine.[3]

It is not altogether clear to what degree the action of the adrenocortical steroids on renal salt excretion is "permissive"[24] and to what extent they directly mediate the degree and duration of sodium retention following withdrawal of blood from the "effective" central circulation. It seems at least possible that the rôle of the mineralcorticoid aldosterone is a direct one; its endogenous excretion rises promptly after hemorrhage[38] or sodium restriction,[40] and its effects upon the excretion of sodium and potassium vary in proportion to the amount injected.[58] Although it has been suggested that altered tubular reabsorptive behavior resulting from increased mineral-

corticoid secretion represents a *chronic* adjustment to circulatory states requiring sodium retention, whereas *acute* changes in sodium excretion are accomplished by intrarenal hemodynamic adjustments,[44] there seems no reason why hormonal adjustments might not also be almost as rapid and immediately responsive to bodily needs as are changes in blood flow. The body manufactures many hormones whose action is immediate and may be short lived; pitressin, epinephrine, and insulin are examples. Huffman, Wilson, Clark, and Smyth have recently accumulated data which show that within 15 minutes after an infusion of 9α-fluorohydrocortisone sodium excretion diminishes markedly;[32, 68] this suggests the possibility of direct adrenal participation in a number of acute renal adjustments, such as the antinatriuresis of quiet standing, of limb congestion, or of hemorrhage.

INTERRELATIONSHIP BETWEEN HORMONES AND HEMODYNAMIC FACTORS IN SODIUM RETENTION AND EDEMA FORMATION

It is difficult in most clinical situations to place the entire responsibility for salt and water retention upon the salt-retaining adrenal hormones. The proper hemodynamic background appears to be essential for the prolonged retention of sodium which results in edema formation—the edema "has to have some place to go." Primary hypersecretion of aldosterone is usually characterized not by edema but by depletion of body potassium.[10] Administration of excessive amounts of desoxycorticosterone to normal dogs produces not edema or ascites but a condition resembling diabetes insipidus.[60]

The interplay between hemodynamic and hormonal factors necessary for edema formation is nowhere better illustrated than in the painstaking experiments of Davis, Howell, and Southworth.[12] Constriction of the inferior vena cava in the thorax produces massive ascites in normal dogs allowed access to salt. Adrenalectomy results in complete loss of the ascites, through progressive renal excretion of salt and water, after which the signs of adrenal insufficiency supervene. Administration of a normal maintenance dose of DOCA halts the renal losses of sodium and will *maintain* ascites, but despite an appropriate hemodynamic background ascites does not *develop* and increase in severity until DOCA is given in twenty to fifty times the normal maintenance dose. Progressive sodium retention and the development of ascites in this species apparently require that the proper circulatory background be coupled with excessive adrenal secretion. On the other hand, Blackett has reported a case of congestive heart failure with edema occurring in an untreated Addisonian;[8] there may, however, have been some residual adrenal secretory activity in this patient.

The rôle of hydrocortisone and of cortisone with respect to sodium excretion appears to be "permissive"; that is, their presence enhances but does not necessarily mediate stimuli to the excretion or retention of sodium. Administration of cortisone restores the normal diurnal excretory pattern of electrolytes in subjects with Addison's disease and improves their ability to retain sodium in the seated posture and to excrete it when supine.[52] Figure 1 shows the results of applying congesting cuffs to the thighs of a patient who had a bilateral total adrenalectomy and was maintained on 2 mg. of DOCA and 50 mg. of cortisone per day. Despite a presumably constant level of circulating adrenal steroids, salt and water excretion fell promptly when the cuffs were applied and rose toward normal when the sequestered blood was allowed to return to the central circulation. Inulin clearance fell slightly during the period of congestion but returned to control levels after the cuffs were released at a time when sodium excretion was still depressed.

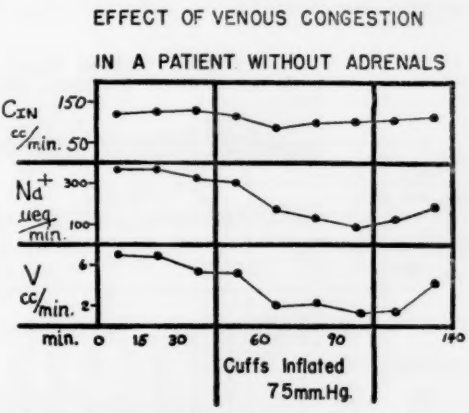
EFFECT OF VENOUS CONGESTION
IN A PATIENT WITHOUT ADRENALS
C_{IN} cc/min. 150 50
Na^+ μeq/min. 300 100
V cc/min. 6 2
min. 0 15 30 60 90 140
Cuffs Inflated 75mm.Hg.

FIG. 1. Trapping blood in the legs of a patient without adrenal glands but maintained on DOCA and cortisone produced a fall in sodium excretion associated with a slight decline in GFR.

VENOUS CONGESTION DURING MANNITOL DIURESIS

Some of the above information is especially pertinent when considering the sequence of events which occurs when blood is trapped in the limbs of normal and sympathectomized subjects during mannitol diuresis. Because venous congestion of the legs is always accompanied by antidiuresis in normal subjects, the calculated renal clearances of inulin and para-aminohippurate as well as other substances are especially susceptible to artifactual depression during this maneuver. In order to overcome this difficulty, a moderate osmotic diuresis can be induced with mannitol which will prevent any great depression of urine flow. When congesting cuffs are applied to the legs of a normal subject under these circumstances, there is an abrupt and progressive decline in the urinary sodium

concentration and sodium excretion associated with a less dramatic fall in glomerular filtration rate and renal plasma flow. This is illustrated in Figure 2. When the same procedure is carried out on a patient who has had a lumbodorsal sympathectomy (Fig. 3), glomerular filtration rate and renal plasma flow do not change, suggesting that effectual renal denervation has indeed been accomplished. Nevertheless, the concentration of sodium in the urine and the renal excretion of sodium promptly decrease, continue to decline during the period of cuff congestion, and start

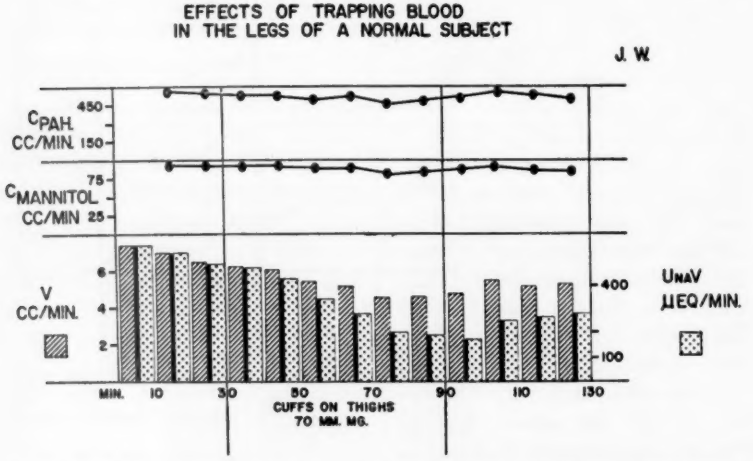

FIG. 2. Venous congestion of the legs of a normal subject *during mannitol diuresis* provokes a greater fall in the excretion of sodium than in the excretion of water. There is a slight drop in GFR and RPF. Mean arterial pressure (not shown) usually does not change during this maneuver.

to return towards normal only when the cuffs are released.[18] In these experiments, as in analogous ones in dogs by Goodyer and Jaeger,[27] possible explanations for the observed retention of sodium might be: (a) intrarenal redistribution of blood flow and filtration rate; and/or (b) the action of rapidly acting and quickly dissipated humoral agents upon the renal tubules.

THE NATURE OF THE "VOLUME RECEPTOR"

Whether sodium retention is accomplished by hormones acting upon renal tubules, by nervous impulses to renal vasculature or renal parenchyma, or by intrarenal adjustments of pressure and flow, the nature and sites of action of *afferent* stimuli to renal excretion or retention of

sodium constitute one of the more important unanswered problems about fluid metabolism today. It is obvious from the very existence of clinical states characterized by edema that the kidneys are not sensitive to the total amount of extracellular fluid. It would seem more likely, perhaps, that they respond to some fraction of the extracellular fluid volume—

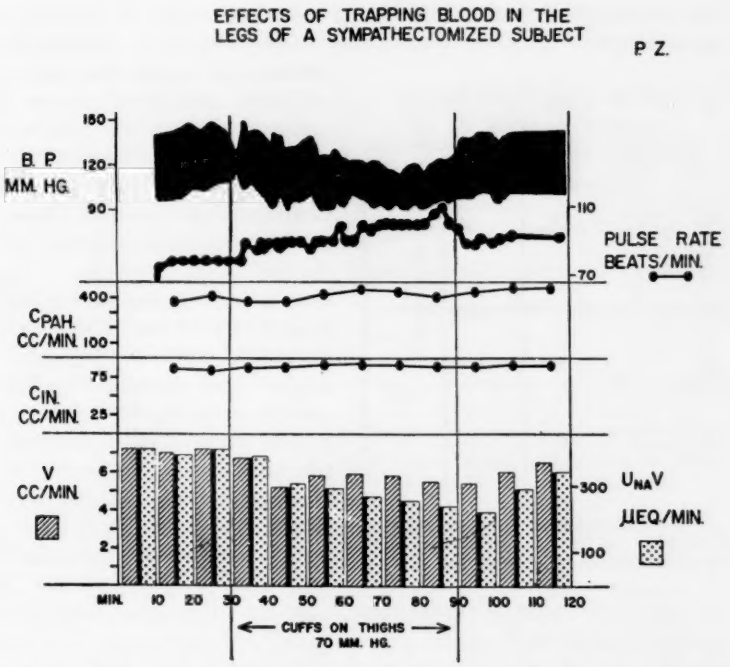

FIG. 3. When blood is trapped in the legs of a sympathectomized subject undergoing mannitol diuresis, sodium excretion falls (in association with a drop in mean arterial pressure) although renal blood flow and glomerular filtration rate are not diminished.

that portion possibly whose function it is to stir and mix the body fluids and to be distributed to all parts of the body. As Peters suggested in 1935,[47] the kidneys behave in many situations as though they were peculiarly sensitive to some function of the volume of circulating blood. It is apparent also from purely clinical considerations that the total volume of blood is not as important to the kidneys as the way it is distributed inside the vascular system. Congestive heart failure is a clinical case in point. Although earlier measurements of plasma volume, using dyes bound to albumin, probably

overestimated the volume of circulating blood in congestive heart failure,[48] it is clear from studies employing tagged red cells that heart failure may be characterized by avid renal retention of sodium at a time when blood volume may be normal or high.[14] The same is true in patients with hepatic cirrhosis and portal hypertension[15] and in patients or animals with large arteriovenous fistulas.[19, 21]

A normal standing subject is in many ways the paradigm of these clinical states of sodium retention. When a man stands erect, venous and capillary pressures in the legs rise, transudation from the blood stream increases, plasma volume falls, blood pools in the large veins and there is compensatory vasoconstriction of arterioles and venules. There is a fall in pressure in the great veins of the thorax, cardiac output usually declines, and arterial pulse pressure diminishes, though mean arterial blood pressure may not decrease. Renal retention of sodium can be demonstrated within the first 20 minutes after the erect posture is assumed. This occurs even when contraction of total blood volume (though not its redistribution) is prevented by simultaneously infusing albumin.[37] Exactly the same sequence can be demonstrated in subjects in the supine position by trapping blood in the limbs with congesting cuffs. Normal persons promptly excrete a load of saline solution given intravenously when they are in a supine position; seated or standing subjects do not, nor do patients in congestive failure. In attempting to fit these and other facts to a single hypothesis, the cranial cavity, the "cephalad portion of the body," the great veins and cardiac atria, and the arterial pressoreceptors have all been proposed as possible sites for the initiation of afferent impulses designed to modify renal excretion of salt or water.

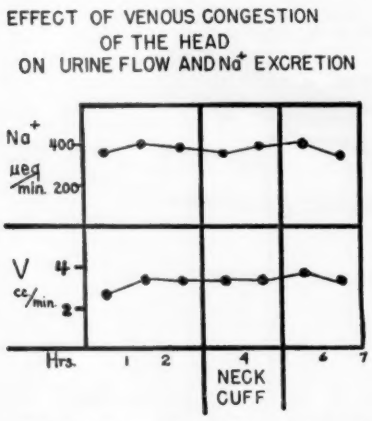

FIG. 4. Inflation of a cuff about the neck to 25 mm. Hg for one hour had no effect on sodium excretion or urine flow.

The idea of a cranial "volume receptor" is a simple and attractive one. It is consonant with the facts that the erect posture is associated with the retention of salt and water and recumbency with diuresis, though not necessarily with the observation that sodium retention occurs with obstruction of the superior vena cava[21] as well as in congestive heart failure, two con-

ditions characterized by the elevation of cerebral venous pressures. Harrison and his collaborators produced an increase in the excretion of salt when congesting cuffs were inflated about the necks of normal semirecumbent subjects.[62] These findings were not confirmed by others[45] or ourselves. Figures 4 and 5 demonstrate that over a period of 30 to 60 minutes sodium excretion is not altered by venous congestion of the head produced either by a pneumatic cuff or by head-down tilting. Moreover, the sodium retention which normally accompanies quiet standing or venous congestion of the legs is not prevented by simultaneous elevation of cerebral venous pressure (Fig. 6). Alterations in cerebrospinal fluid pressure, cerebral venous pressure, or cerebral venous distention within the physiological range were not noted by Fishman to influence renal excretion of sodium in anesthetized dogs.[24] Changes in venous pressure in the head therefore do not appear to play a major rôle in regulating sodium excretion under usual circumstances.

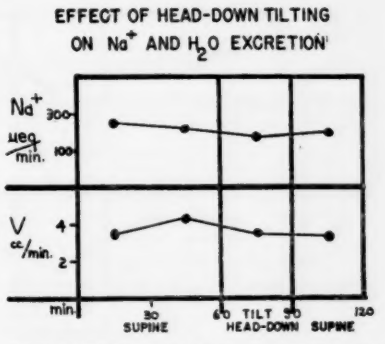

FIG. 5. Tilting head downward at an angle of 45° for 30 minutes had no effect on sodium excretion or urine flow.

The fact that sodium retention regularly accompanies the peripheral dislocation of blood into congested veins has led to speculation that neural impulses from the distended segments of veins might provoke the kidneys to reabsorb salt. Judson, Hatcher, Halperin, and Wilkins showed that the renal effects of venous congestion in a supine subject were secondary to the withdrawal of blood from the general circulation.[35] Sodium was not retained when a volume of blood equal to that being trapped in the limbs was infused simultaneously.

That cardiac output might be directly related in some way to renal sodium excretion was suggested by Borst,[9] primarily because it seemed to be so related in patients with gastrointestinal hemorrhage and congestive heart failure. It is difficult to visualize how the kidneys could be accurately apprised of the minute volume of cardiac output per se, unless it were reflected in the renal blood flow. Cardiac output is *inversely* related to salt excretion in normal persons performing muscular exercise in the standing position,[37] in patients and dogs with arteriovenous fistulas[19, 21] and in all types of "high-output" cardiac failure.

Most of the states in which renal retention of sodium is prominent are associated with either a diminution of total blood volume or a sequestration of blood in peripheral veins. There is one situation in which this is not the case. Infusions of concentrated solutions of albumin into normal subjects raise cardiac filling pressure, increase cardiac output, and expand blood volume at the expense of interstitial fluids. This is, paradoxically, accompanied by a *decrease* in the renal excretion of sodium.[28] These facts have suggested that the kidneys might be sensitive in some way to a contracted interstitial fluid even in the presence of a swollen vascular volume, or that there might be a receptor organ within the vascular tree responsive to alterations in oncotic pressure.[49, 68] Conceivably this "receptor" might be in the cranial cavity. Infusions of concentrated albumin solutions into the carotid artery of a dog do not, however, provoke renal retention of sodium (Table 1). Although an elevation in the level of serum albumin in blood perfusing the kidney might be expected to exert a direct effect in diminishing slightly glomerular filtration and/or increasing tubular reabsorption of water and salts, Goodyer and Glenn[28] observed this to occur only inconstantly when 25 per cent albumin was injected directly into the left renal artery. In many experiments of Goodyer and Glenn, however, renal excretion of sodium was already at a minimum before albumin was injected. For this and other reasons the possibility remains an important one that the sodium-retaining action of concentrated albumin in normals is a direct intrarenal effect.

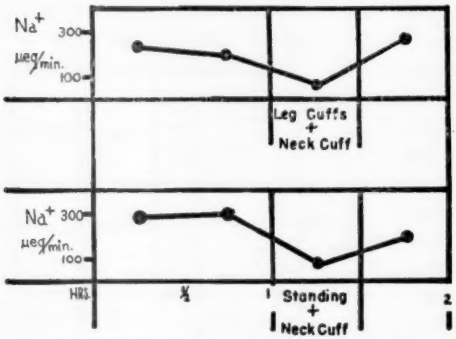

FIG. 6. Failure of head congestion to prevent retention of sodium. In two separate experiments on the same subject, inflating a cuff about the neck to 25 mm. Hg did not prevent the fall in sodium excretion which normally accompanies venous congestion of the legs or quiet standing. The subject was supine during the CONTROL and RECOVERY periods.

If the kidneys do respond to changes in volume (or pressure) in some portion of the vascular system, the question remains, which portion? Observing that immersion of the body in water always produced a diuresis, Bazett deduced that a shift of blood from peripheral venules in the skin to a more central location was responsible.[5] Pressures in the great veins of the

chest, including both atria, are easily altered by relatively small changes in blood volume induced by blood-letting or infusions. For this reason, Gauer, Henry, Sieker, and Wendt suggested that these structures might be the locus of stretch receptors which would initiate renal conservation or excretion of fluid.[25] Negative-pressure breathing distends the intrathoracic vasculature and provokes diuresis in men[26] and dogs.[25] This reaction is also obtained when the left atrium of the dog is distended with a balloon but not when the pulmonary veins are occluded.[20] Positive-pressure breathing causes a decreased excretion of both water and salt;[18] however, the increased urinary flow which sometimes follows negative-pressure breathing is not ac-

TABLE 1. EFFECT OF INFUSING 25 PER CENT ALBUMIN INTO THE CAROTID ARTERY OF AN UNANESTHETIZED DOG

Time min.	*Procedure*	$U_{Na}V$ *uEq./min.*
10	Control	264
22	"	285
33	"	297
43	Infuse 100 cc. 25 per cent	308
52	albumin into carotid artery	320
64	Recovery	330
79	"	332

companied by a diuresis of sodium. Nor does loss of water from the body normally provoke sodium excretion, or a positive balance of water, sodium retention; in fact, the exact reverse is true.[16, 20] For these reasons the importance of atrial receptors in the homeostatic defense of the volume of plasma and extracellular fluid is unclear. They would not explain why sodium and water are retained in congestive heart failure in association with increased distention of the atria and other "low-pressure" areas of the lesser circulation.

Patients and animals with compressible arteriovenous fistulas afford a unique opportunity to study the reaction of the kidneys to abrupt dislocations in the circulation. As has been said of the patient with congestive heart failure,[4] the subject with an arteriovenous fistula is literally bleeding into his large veins. When the fistula is occluded, the arterial tree is emptied more slowly and less completely, and mean arterial pressure rises. Pressures in the great veins, the right atrium, and the pulmonary vessels tend to fall as these regions become less distended with blood. Cardiac output is decreased as the heart rate slows and stroke volume diminishes. At

the same time, there is an abrupt increase in the renal excretion of sodium without concomitant changes in glomerular filtration rate or renal blood flow.[19] When the fistula is opened, arteries become less distended and mean systemic arterial pressure falls, while the great veins become more swollen and pressures in the lesser circulation may increase. As this occurs, renal excretion of sodium diminishes markedly. These data suggest that changes in arterial filling or arterial pressure stimulate changes in the renal handling of sodium.[19, 20] It may be pertinent in this connection to note that patients with arterial hypertension excrete saline loads much more rapidly than do normotensive subjects.[7, 60]

It is interesting to list the circulatory states, chronic as well as acute, in which the kidneys tend to retain sodium. Anemia, hemorrhage, hypoproteinemia, and dehydration are characterized by a decreased total blood volume. During stationary standing, venous congestion of the limbs, partial occlusion of the superior or inferior vena cava, and portal hypertension, filtration from the capillaries is increased and in addition blood is pooled in the peripheral veins away from the general circulation. Constrictive pericarditis, constriction of the pulmonary artery, and congestive heart failure are characterized by distention of the central veins with blood which the heart is unable to pump efficiently into the aorta. In some of the above conditions the volume of blood in the central veins is reduced; in others these vessels are engorged. In all, however, there exists a tendency toward inadequate filling of the systemic arterial tree, either because of a diminished total blood volume or an altered distribution of blood within the vascular system. The latter may have its origin either in pooling or shunting of blood in the periphery or in failure of the heart as a pump. These facts are consistent with the hypothesis that renal excretion or retention of sodium is conditioned by the degree of filling of some portion of the arterial tree. In this view renal retention of salt and water would fall into the same category as a variety of vascular reactions (e.g., the carotid sinus and aortic reflexes) designed to maintain the integrity of the circulation.

The ingenious experiments of Goodyer and Jaeger[37] suggest that some vascular volume, rather than arterial pressure per se, is important in influencing sodium excretion. Arterial pressure was maintained constant in dogs at the level of an outside reservoir of blood to which the animals' arterial system was connected. When a ganglionic blocking agent was injected, blood entered the vascular system from the reservoir without changing arterial pressure; this was followed by an increase in the output of sodium from both innervated and denervated kidneys.

A corollary of the idea of a volume receptor located in the arterial tree is the concept that changes in intravascular volume need not follow passively

changes in intravascular pressure. (It may be worth while to point out that this is equally true of afferent arterial receptors for vascular reflexes; if the heart rate responds chiefly to the level of mean arterial pressure, why does it increase along with a rise in mean arterial pressure in the upright position?) The measurement of the distensibility of the great arterial reservoir *in vivo* presents almost insuperable difficulties. Nevertheless, Remington, Noback, Hamilton, and Gold[81] have adduced evidence, based on measurements of cardiac stroke volume by the Fick method compared with calculations of simultaneous stroke output from pulse pressure measurements, that the distensibility of the aorta may indeed be decreased in congestive heart failure, a condition in which blood is sequestered in the great veins without necessarily producing a fall in arterial blood pressure, while sodium is retained by the kidneys.

The concept of a volume receptor is perhaps too easily verbalized and visualized; its appeal is seductive. Undoubtedly there is danger in looking for simple and concrete answers to complex problems; it is difficult, as Wolf has remarked, to avoid casting one's lot with that of a neat idea. Yet the question of volume regulation is a real one; it cannot be begged by mere descriptions of the filtration and reabsorption of sodium and water by the kidneys. If the Hunt for the Volume Receptor is to proceed on a firmer footing than the Hunting of the Snark, it behooves the careful investigator[84] to observe more stringent standards than that of the Bellman in Lewis Carroll's poem:

> "Just the place for a Snark," the Bellman cried
> As he landed his crew with care,
> Supporting each man on the top of the tide
> With a finger entwined in his hair.
>
> "Just the place for a Snark! I have said it twice.
> That alone should encourage the crew.
> Just the place for a Snark! I have said it thrice:
> What I tell you three times is true."

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THE INFLUENCE OF DISEASE ON THE RENAL EXCRETION OF WATER

The rate of excretion of water by the healthy kidney is determined primarily by the excretion of solutes, the influence of the antidiuretic hormone (ADH), and a presumed hormone-independent mechanism for the renal tubular reabsorption of water. Disease processes influence the flow of urine by imposing restrictions or excesses in relation to these several regulatory factors. It is the purpose of this essay to describe the currently most acceptable formulation concerning the mechanisms involved in the regulation of the excretion of water and to examine within the context of this theory the disabilities imposed by disease. Such an approach may have a dual advantage in that it helps to provide some clarification of the essential pathophysiology of a disease process, and at the same time, it demonstrates certain inadequacies of the theoretical formulation.

The reabsorption of the water filtered at the glomerulus is presumed to be accomplished at three levels within the nephron.[66, 67, 80, 81] The first phase, which occurs in the proximal tubule, is considered to be passive and secondary to the active reabsorption of solutes. The abstraction of solutes creates a dilute tubular fluid, and water then diffuses through the tubular membrane along the established gradient. Thus, the volume of fluid which remains at the end of this operation is clearly dependent on the volume of the original filtrate and the extent to which the solutes have been reabsorbed. Under most circumstances this volume approximates 85 to 90 per cent of the initial filtrate.[73] Although the several solutes are reabsorbed at different rates by separate transport systems and the composition of the fluid is altered thereby, the diffusion of water maintains the total solute concentration isosmotic with the filtrate.[73]

The second phase is ascribed to the distal tubule and is thought to be influenced by the antidiuretic hormone. The hypothesis suggests that, as in the proximal tubule, the active reabsorption of solutes in the distal nephron creates a dilute tubular fluid. However, in the absence of the antidiuretic hormone little or no water is reabsorbed, whereas with maximal ADH activity the reabsorption of water proceeds to the point where the tubular fluid is again isosmotic with the parent filtrate. Presumably, with levels of activity of the antidiuretic hormone intermediate between none and maxi-

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mal, the reabsorption of water will be less than that required to keep the tubular fluid isosmotic with plasma.

Thus, at the end of this second phase a variably hypotonic or an isosmotic fluid reaches an even more distal site (perhaps, the collecting tubule) where water is reabsorbed without solutes. Two limits are imposed on the quantity of water that may be removed in this third operation. The first is a limit in terms of rate such that not more than 5-7 cc. per minute (per 100 cc. of filtrate) can be reabsorbed at this site. The second limit concerns the maximal osmotic gradient that can be achieved between the urine and the body fluids, which is between four and five to one. This final operation is referred to as $T^{c}_{H_2O}$ by Smith and his colleagues. The term refers to that operation which serves to concentrate the tubular fluid by the abstraction of water.

There are data from a variety of sources which support the general framework of this concept. The early micropuncture studies of the renal tubules established the approximate volume of the reabsorbate in the proximal tubules,[72] and reported the essential identity between the vapor pressures of the tubular fluid and the plasma.[73] More recently, Wirz[85] has reported some new observations on the osmotic activity of the water of the fluid obtained from the proximal and distal tubules of the rat during a water diuresis and dehydration antidiuresis. He confirmed the observation that the fluid in the proximal tubule is isosmotic with filtrate. In contrast, however, the fluid obtained from the first portion of the distal tubule is hypotonic to plasma, and in circumstances of a water diuresis this fluid becomes progressively more dilute along the distal tubule. Fluid obtained during a dehydration antidiuresis is noted to become isosmotic with plasma but it never exceeds this level of total solute concentration. Since this latter observation was recorded at a time when the bladder urine was concentrated, it is apparent that water must have been abstracted without (or at least in excess of) solutes at some site beyond the distal tubule.

Shannon has observed the excretion of concentrated urines in dogs with experimental diabetes insipidus when they were subjected to dehydration.[82] It could not be concluded with safety that this occurred independently of ADH since it is essentially impossible to state with certainty that there is ever complete inability to secrete this hormone in experimental or clinical diabetes insipidus. Berliner and Davidson[8] recently reported an investigation dealing with this problem in which there seems to be little question that they were able to promote the excretion of a urine hypertonic to plasma despite the lack of evidence of secretion of the antidiuretic hormone. They promoted a water diuresis in dogs and the characteristics of the urine from each kidney was independently examined. When a steady rate of excretion of a hypotonic urine had been observed, the renal artery on one side was

compressed so as to reduce the rate of filtration at the glomerulus on that side. This, in turn, promoted a drastic reduction in the rate of excretion of solutes on the ipsilateral side which was accompanied by the excretion of a urine in which the total concentration of solutes exceeded that in the plasma. The urine from the contralateral side remained dilute and appeared to support the assumption that the procedure did not cause a secretion of ADH. The elaboration of a concentrated urine on the experimental side is ascribed to the accession of an unusually small volume of dilute fluid to the $T^{C}_{H_2O}$ site. The abstraction of solute-free water at this locus was apparently adequate to concentrate the urine to a level higher than the plasma.

Not only does this hypothesis account for the excretion of a urine more concentrated than filtrate in the absence of ADH, it also allows for the excretion of a minimally concentrated urine despite maximal activity of the antidiuretic hormone. This is, of course, observed when the rate of excretion of solutes is high and is referred to as a solute or osmotic diuresis.[54] This obtains whenever the reabsorption of solutes is less complete than usual. This may be due to an unusually high concentration in the filtrate of some normal component such as glucose or urea, the reabsorption of which is limited, or a foreign substance such as mannitol which the tubules are unable to reabsorb, or to the administration of some pharmacological agent that blocks the active reabsorption of one of the usual components such as sodium.[78, 79] This is frequently, but not necessarily, associated with an increased delivery of solutes and water from the proximal to the distal tubule. The net effect of the second phase operation under maximal ADH activity will be the delivery of an unusually large volume of isosmotic tubular fluid to the $T^{C}_{H_2O}$ site. This last operation is now limited by a maximal rate and as the excretion of solutes is increased, the final bladder urine represents the effect of the abstraction of a constant volume of water (i.e., $T^{C}_{H_2O}$) from an ever-increasing volume of isosmotic fluid. It is clear that the concentration of this urine will approach the concentration of filtrate as the rate of excretion of solutes is increased.

This formulation of the theory of the renal tubular reabsorption of water as developed by Smith,[86, 87] Wesson and Anslow,[80, 81] Page and Reem,[49] Ladd,[87] and Zak, Brun, and Smith[97] was derived in great measure from a study of the characteristics of solute diureses. In these considerations the urinary water is treated as two fractions or clearances spoken of as osmolar water and free water:

$$V = C_{Osm} + C_{H_2O}$$

where V is the rate of excretion of water per minute, and C_{Osm} and C_{H_2O} are the clearances of osmolar and free water, respectively. The osmolar

water is that volume which could contain the solutes, excreted per minute, at a concentration identical with that of the plasma filtrate. Thus, C_{H_2O} has a positive value only when the urine is hypotonic and has a negative value when the urine is concentrated in excess of that of the filtrate. A negative free water clearance may be equated with the volume of water abstracted from the isosmotic fluid in the third operation spoken of earlier as $T^C_{H_2O}$. It may be helpful to visualize these definitions in symbolic terms.

$$C_{Osm} = \frac{U_{Osm}}{P_{Osm}} V$$

In this expression U_{Osm} and P_{Osm} refer to the osmotic activity of the water of urine and plasma respectively. Since

$$V = C_{Osm} + C_{H_2O},$$
$$C_{H_2O} = V - C_{Osm}$$

In order to assign a positive value to $T^C_{H_2O}$ (C_{H_2O}) when the urine is hypertonic, the signs are changed and

$$T^C_{H_2O} = C_{Osm} - V$$

A recent report by DeWardener and Del Greco[15] described the alterations in urine concentration consequent to the administration of infusions of mannitol to subjects with diabetes insipidus. In one group of studies the subjects received small quantities of pitressin varying from 1 to 13 mUnits/hour, and in another, smaller series the subjects received no pitressin. In the former group the urine concentrations fell from 350-400 mOsm./L. to hypotonic levels, and in the others the concentration of the dilute urine increased toward that of the filtrate, and the clearance of free water was enhanced as well. They interpret these data to mean that the fluid delivered to the $T^C_{H_2O}$ site was hypotonic. There is no argument with this interpretation. However, their inference that this is in disagreement with the Smith hypothesis has no validity since this formulation anticipates the delivery of an isosmotic fluid to the third phase operation only under circumstances of *maximal* ADH activity. This condition did not obtain in these studies. The data are, in fact, completely compatible with the mechanism suggested to be responsibile for dilution of the urine, namely, the reabsorption of solutes in excess of water in the distal tubule when ADH activity is absent or incomplete.

There are certain data that imply that this formulation is at least incomplete. For example, if the quantity of $T^C_{H_2O}$ were, in fact, limited by only two parameters, rate and a maximal osmotic U/P ratio, then the maximal

U/P ratio should obtain whenever urine flow is less than the maximal $T^C_{H_2O}$ (referred to as $Tm^C_{H_2O}$). This appears to be at variance with the data, however, and maximal osmotic U/P ratios are only observed when the rate of excretion of solutes is quite small.

The gradual rather than abrupt change from a maximal U/P ratio as solute excretion increases under conditions of maximal ADH activity has not been properly explained. Robinson[56] raised the question as to whether the interrelationships between the rate of flow of urine and the tubular dimensions were such as to create a situation where inadequate time was allowed for diffusion equilibrium to be established except at the lowest rates of flow. He made calculations based on a formula which relates the transfer of heat to a fluid which flows through a pipe along which there is at one point a sharp change in temperature. The applicability of this problem to that of the reabsorption of water is based on the speculation that a group of tubular cells with a high osmotic pressure might reside at a particular locus in the tubule and be responsible for the abstraction of water in virtue of the difference in the osmotic activity of the cellular and tubular water. Robinson[56] employed a fair number of assumptions in order to make appropriate substitutions in the heat transfer formula and concluded that it is unlikely that inadequate time for diffusion equilibrium with modest urine flows could account for the failure to elaborate a urine with a maximal osmotic U/P ratio. Nevertheless, this possibility deserves further consideration, as does any other that may serve to resolve this dilemma.

In the studies reported by Zak, Brun, and Smith[57] considerable attention is devoted to the character of the slope of the regression line of C_{Osm} on V. If the $T^C_{H_2O}$ mechanism is saturated, and if the antidiuretic hormone assures the delivery of an isosmotic fluid to the $T^C_{H_2O}$ site, then each increment of V should be equal to the increment of C_{Osm} and the slope of the regression line should equal 1.0. In fact, the values for the slopes of these lines varied from 0.863 to 1.168, but in any individual instance there was such a degree of consistency in performance that Zak and his colleagues were inclined to ascribe physiological significance to the deviation from a slope of 1.0. If this interpretation is valid and $Tm^C_{H_2O}$ is constant, a slope greater than 1.0 implies that the increment of fluid which escaped reabsorption in the distal tubule had an osmotic U/P ratio greater than 1.0, and would imply that water had been reabsorbed in excess of solutes in the second phase of the operation. In contrast, a slope less than 1.0 implies that the reabsorption of water did not proceed to the isosmotic limit in the distal tubule despite maximum activity of the antidiuretic hormone. One alternative interpretation is that $T^C_{H_2O}$ is not constant. Still another explanation is that the slope

does not deviate significantly from 1.0 and that the apparent deviations are, somehow, artificial. If one examines the arithmetic expression for C_{Osm} which is U_{Osm}/P_{Osm} V, it is apparent that the U/P osmotic ratio can be made greater or less than 1.0 by altering the value for P_{Osm}. If P_{Osm} were to be increased rapidly and the reflection of this in U_{Osm} were to lag, the ratio would be less than 1.0. There were six examples of slopes less than 1.0 in the 21 experiments reported by Zak *et al.* It is interesting to note that five of these were in subjects whose range of P_{Osm} during the study was nine or more. It would be reasonable to infer that a sharp rise in P_{Osm} would have been more likely to occur in studies in which the total range of P_{Osm} was large. An analysis of the data with the assumption that the slopes are approximately normally distributed reveals that the average slope does not differ significantly from 1.0. It is difficult to select the more appropriate interpretation. If a deviation of the slope from 1.0 could be taken seriously, this would be a most useful tool in the study of the pathophysiology of certain disorders characterized by an inability to concentrate the urine. It might serve to provide a basis for discriminating between the ADH-independent and dependent mechanism of water reabsorption as the source of the disorder. This would be particularly true if serial studies revealed significant changes in the slope as a lesion was induced or repaired.

Despite these problems this hypothesis is most useful and accounts best for most of the physiological data. The few inadequacies probably imply an incomplete rather than an incorrect formulation.

Although the structure of the antidiuretic hormone has been established by du Vigneaud[10] as an octapeptide amide, there are many problems concerned with it which remain unanswered.[70] These include information concerning all of the stimuli that are responsible for its secretion,[1, 6, 9, 12, 27, 28, 39, 45, 46, 60, 71, 74, 75] the mode of transport and storage of the hormone,[61] the relationship between the quantity of the hormone and the intensity of the response,[32] the locus of its action,[67] and the nature of its mode of action.[56] Partial answers to some of these questions are available and have been presented in other reviews.[41, 74, 75]

The influences of diseases as conditioning factors modifying the rate of excretion of water are many, and they vary from rather simple situations to those which are more or less subtle and not completely understood.[73] Perhaps the most simple of all is diabetes insipidus which is the clinical expression of partial or total insufficiency of the antidiuretic hormone.[7, 11, 52] The disease may be due to a variety of lesions which destroy or interfere with the function of some part of the supra-optico-hypophyseal pathway.[21, 22] The list includes tumors, infections such as syphilis and encephalitis, tuberculo-

sis, cerebrovascular disease, and trauma. Some instances appear to be inherited,[39, 40] and others are never defined as to etiology. The clinical features include the anticipated polyuria and polydipsia, as well as the symptoms that may be related to the characteristics of the disease process responsible for the disturbance of the integrity of the supra-optico-hypophyseal system. The questions concerning the primacy of the polyuria,[1, 2, 75] and the influence of other endocrine disorders on the characteristics of diabetes insipidus are discussed elsewhere.[89]

It is, of course, not surprising that renal disease may be associated with disturbances in the rate of excretion of water and the ability to concentrate the urine. There are three types of renal disease that are characterized by an inability to concentrate the urine and which cannot be explained as examples of the consequences of solute diureses. In these instances the urine is dilute, the disorders may mimic diabetes insipidus, and they appear to be examples of end-organ failure. The first of these, referred to as nephrogenic diabetes insipidus,[84] is inherited, and its manifestations are noted early in life. The kidneys of these patients are unresponsive to pitressin. The nature of the insensitivity to the antidiuretic hormone is not understood. A second group is acquired and has been referred to as "water-losing nephritis."[17, 59] These patients who have had obstructive uropathy or multiple myeloma developed thirst, and polyuria, and were also unresponsive to pitressin. It is of especial interest to report that the kidney tissue of one of the two patients reported by Roussak and Oleesky[59] was examined by Darmady[14] using the technique of nephron micro-dissection and lesions were found in the collecting tubule and to a lesser extent in the distal tubule as well.

Several reports have appeared concerning the inability to concentrate the urine appropriately in patients with potassium depletion.[28, 55, 58, 62] This has been studied and confirmed in experimental potassium depletion in rats and there appears to be a relationship between the degree of potassium deficiency and the intensity of the functional disability. Dr. Jean Oliver,[48] utilizing the techniques of micro-dissection, is investigating these experimentally depleted kidneys; the most definite and consistent lesion appears to be in the collecting tubules. The association of an anatomical lesion in the renal tubule at a specific site with a particular functional defect certainly cannot be interpreted as proof that that particular area is the locus of the physiological operation of which the malfunction has been demonstrated. At the same time the speculation that the two observations may be correlated is intriguing. To the extent that these inferences concerning topographic pathophysiology are valid, the Smith formulation is supported.

It was stated that these three types of renal disease were accompanied by a disability in the renal concentrating mechanism which could not be

ascribed to the consequences of a solute diuresis. This latter, however, may be an important factor in the inability to concentrate the urine in chronic renal insufficiency. A completely satisfactory explanation must satisfy the usual sequence which is represented first by an inability to concentrate the urine while the ability to dilute the urine appears to persist, ending, ultimately, in the inability either to concentrate or dilute the urine with the production of a fluid that varies little from the concentration of plasma filtrate. So long as the kidneys were to retain the ability to excrete a dilute urine but were unable to produce a concentrated urine it would be possible to suggest that the defect is simply an inability of the appropriate renal tubular cells to respond to the antidiuretic hormone. However, when the stage of isosthenuria is reached, this hypothesis is incomplete.

Several groups have suggested that the apparent defect in concentration as reflected in the maximal U/P osmotic ratio is misleading. They suggest the hypothesis that chronic renal insufficiency is primarily characterized by a reduction in the total number of functioning nephrons and that those that remain are healthy, rather than that there is greater or lesser partial destruction of almost all the nephrons.[30, 51, 52] The former hypothesis has certain important implications. Among these is the assumption that each remaining nephron is functioning at a higher than normal level in certain respects. There seems little doubt that the volume of filtrate formed per glomerulus is increased when renal tissue is experimentally reduced. If the increase in the rate of filtration at the glomerulus exceeds any increase in the proximal tubular reabsorption of solutes, a larger volume of fluid will gain access to the distal tubule. Even if solute reabsorption is enhanced at this site, the rate of excretion of solutes per nephron will be augmented. This is the characteristic of a solute diuresis and reference to the earlier discussion may make it clear why the urine would fail to be highly concentrated despite maximal secretion of, and renal tubular response to, the antidiuretic hormone. This abnormality will, of course, become more profound when despite an increase in the rate of filtration at the glomerulus per nephron the total number of functioning nephrons decreases to the point where the concentration of urea is elevated. Under this circumstance the load of filtered solutes per nephron is markedly increased by virtue both of an increased filtration rate and an increased concentration of solutes in the filtrate. Since the reabsorption of urea is limited, it is particularly responsible for the increased solute diuresis, the intensity of which may now be sufficient to insure a urine concentration which is always close to that of the filtrate.

Values for C_{Osm}, $T^{C}_{H_2O}$, and glomerular filtration rate were reported in a study of the renal concentrating mechanism in patients with kidney

disease by Baldwin, Berman, Heinemann, and Smith.[8] They measured the osmotic U/P ratio in control periods and then administered a 10 per cent solution of mannitol. They observed that the value for $T^C_{H_2O}$ per 100 cc. of filtrate is preserved reasonably well until renal insufficiency had progressed to the point where the filtration rate was less than 60 cc. per minute. In these more advanced states of renal disease the value for $T^C_{H_2O}$ per 100 cc. of filtrate was diminished. They interpreted this as evidence for a specific tubular defect. However, this need not necessarily be the case for if the filtration rate per nephron continues to increase as fewer nephrons remain to function, and if the $T^C_{H_2O}$ mechanism was unable to hypertrophy any further, the ratio of $T^C_{H_2O}$ per 100 cc. filtrate would be diminished, and this would give the erroneous impression of a specific tubular defect. This might merely represent an example of a disproportionate increase in renal functions with a consequent glomerulo-tubular imbalance.

These same considerations must impose some limitation on the ability to excrete a dilute urine. However, in the early stages, the abstraction of solutes without water in the distal tubule (in the absence of ADH) may increase sufficiently to insure some dilution of the final urine. Although the changes should be present in both directions (dilution and concentration), they might *appear* to be less impressive in terms of dilution. This may be a semantic problem since one tends to consider a maximal urine concentration of 700 mOsm./L. as a greater defect than a minimal urine concentration of 150 mOsm./L. More data concerning the relationships between the disorders in concentration and dilution of the urine in the course of chronic renal insufficiency are needed to evaluate this hypothesis. Since the specific gravity of the urine cannot be reliably used as an estimate of total solute concentration, techniques employing one of the colligative properties are essential for this purpose.

Inability to excrete large volumes of a dilute urine in response to a water load is a characteristic of certain diseases and clinical disorders as different as cirrhosis of the liver with ascites, dehydration with salt depletion, anterior pituitary insufficiency, cardiac failure with edema, adrenal cortical insufficiency, and subsistence on a dietary regimen low in proteins. In several of these conditions some defect specifically characteristic of that disorder has been cited as the responsible mechanism for the abnormality in the renal excretion of water. In cirrhosis of the liver it has been attributed to inadequate inactivation of the antidiuretic hormone by the diseased liver;[50, 58, 68] in cardiac failure[59, 65] it has been ascribed to an increased sensitivity to ADH; in anterior pituitary insufficiency it has been assigned to the loss of a presumed "diuretic" hormone;[10] and in adrenal cortical in-

sufficiency it has been ascribed to the lack of a specific counteracting influence of the adrenal cortical hormones with respect to the antidiuretic hormone.[20, 36] The hypothesis which has been presented concerning the regulation of the rate of excretion of water may be boldly challenged by the search within its framework for some uniform pathophysiological sequence responsible for the abnormality among these unrelated disorders.

Increased quantities of antidiuretic substance have been found in the urine and blood in some investigations of these patients.[4, 43, 57] The physiological specificity of the antidiuretic substance may be questioned, but even if it is granted that this material does, in fact, represent ADH, it does not necessarily follow that an increased quantity of antidiuretic hormone is responsible for the impaired excretion of water in all instances. Nevertheless, it may very well be one of the mechanisms in some instances. The stimulus which might be responsible for the secretion of the antidiuretic hormone in these disorders is not clear. The principal physiological stimulus for the secretion of ADH is an increase in the effective osmotic pressure of the fluids bathing the osmo-receptor in the anterior portion of the hypothalamus.[71] In contrast, in many instances of these disorders the antithesis of this obtains and is reflected in a depressed concentration of sodium in the serum. There is evidence, however, that a deficit of volume in some critical segment of the vascular or extravascular compartment,[8, 19, 31, 50, 64] or some secondary consequence of this deficit, may also serve as a stimulus to the secretion of the antidiuretic hormone. It is clear that certain of these conditions might share the common denominator of a volume deficit. This is most obvious in ordinary salt depletion dehydration, adrenal cortical insufficiency, and, perhaps, in some instances of anterior pituitary insufficiency as well. The *accumulation* of ascites in patients with cirrhosis implies that the rate of transudation of fluid into the peritoneal cavity is in excess of its reabsorption. This, in turn, predicts a net redisposition of volume between the plasma and this extravascular collection in favor of the latter. The estimation of blood volume may suggest that there is no hypovolemia, but this must be interpreted in relation to the fact of a considerable pooling of blood in the dilated portal venous system and in this circumstance the "effective" blood volume would be considered to be diminished. The fact that there is *hyper*volemia in congestive heart failure need not exclude this same hypothesis. It is reasonable to suggest that the redisposition of blood volume in patients with congestive heart failure may be such as to establish a diminished arterial volume despite an increase in total blood volume. A receptor in the arterial tree responsive to some function of a diminished volume (pressure, flow, etc.) might then be stimulated in patients with cardiac decompensation. Thus, if there is an increased secretion of anti-

diuretic hormone in these clinical disorders, it is reasonable to infer that this has been promoted in response to a physiological stimulus arising in the sequence of events initiated by the primary disorder itself. In support of these suggestions the studies of Leaf and Mamby[38, 43] and others should be cited, since these investigations demonstrated increased quantities of antidiuretic substance in the blood and urine of animals with salt depletion dehydration despite hyponatremia. An increase in plasma volume may promote a diuresis of water in hydropenic subjects which is interpreted as evidence that an expansion of plasma volume suppresses the secretion of ADH.[77] Mirsky and his collaborators[44] have demonstrated that there is an increase in antidiuretic activity of the serum of adrenalectomized animals only when they are allowed to become dehydrated.

Strauss, Birchard, and Saxon[68] noted an increase in the flow of a more dilute urine in some water-loaded patients with cirrhosis and ascites when they were given ethyl alcohol (in a palatable form) to drink. Since alcohol induces diuresis in virtue of suppression of the secretion of the antidiuretic hormone,[18, 35, 69] the data were interpreted as suggesting that the responsive patients were secreting ADH as a consequence of a nonosmotic stimulus.

The suggestion that patients with cirrhosis of the liver with ascites and those with congestive heart failure have an increased sensitivity to ADH is discredited by several investigations.[29, 47, 65, 88] These studies report a normal interval between the administration of a water load and the time when the maximal rate of excretion of water was achieved, a normal response to the administration of pitressin, and the usual interval between the administration of the agent and recovery from same when the dose of pitressin was at reasonably physiological levels.

Although the failure to excrete large volumes of dilute urine in these disorders may, then, be associated with an increased secretion of ADH due to the operation of a normal response to an unusually intense physiological stimulus, it would appear that another mechanism must be operative in some circumstances where the defect persists in the absence of any evidence of antidiuretic hormone activity.

Reference to the description of the several operations involved in the reabsorption of water by the renal tubules will serve to emphasize the close dependence of the rate of excretion of water on the rate of excretion of solutes.[84] Sodium salts and urea represent large fractions of the excreted solutes, and, hence, any factors that affect their rate of excretion must have a profound influence on the rate of excretion of total solutes. A poor protein intake, an intense stimulus for the reabsorption of sodium and its salts, and a decrease in the glomerular filtration rate are the factors which will condition solute excretion. Moreover, the two latter factors are likely to in-

crease the reabsorption of sodium salts in the proximal tubule. This, in turn, must restrict the volume of fluid that can gain access to the distal tubule. Since the maximal rate of excretion of water, even in the absence of ADH, cannot exceed the volume delivered to the distal tubule less the volume of $T^{C}_{H_2O}$, it is clear that a diminished volume of filtrate and the avid reabsorption of salt (and water) in the proximal tubules must impose serious limitations on the rate of excretion of urine. These conditions are common in salt depletion dehydration, congestive heart failure with edema, and in cirrhosis of the liver with ascites. A diminished rate of excretion of urea in subjects subsisting on a low protein diet is responsible for a reduced water diuresis in a similar fashion. The diminished nitrogen metabolism and the factor of a decreased filtration rate in anterior pituitary insufficiency are probably responsible for the water defect in that disorder.

The situation in adrenal cortical insufficiency is, at first glance, more difficult to resolve, since, if anything, the excretion of solutes, as salt, is enhanced. Moreover, the inability to achieve a proper diuresis of water persists after hydration has been restored so that it cannot be ascribed solely to the secretion of the antidiuretic hormone. If the reabsorption of sodium salts which is implemented by the salt-retaining hormone of the adrenal cortex were confined to the distal tubule, the failure to diurese properly in adrenal insufficiency could be readily explained along lines similar to those just presented. The filtration rate is depressed in adrenal cortical insufficiency and this hemodynamic alteration (and possibly others as well) would tend to promote an enhanced reabsorption of sodium salt in the proximal tubule.[10, 24, 25] To the extent that these factors encourage the conservation of salt by the proximal tubular cells, a smaller volume of isosmotic fluid will gain access to the distal tubule. In this fashion the same restrictions are imposed on the excretion of water described in relation to cirrhosis, heart failure, salt depletion, small urea excretion, and anterior pituitary insufficiency. There are, however, two differences. If the assumption that the salt-retaining hormone acts on the distal tubule is valid, the salt that did reach the distal tubule would escape reabsorption and be excreted in the urine. Secondly, if the dilution of the urine is dependent on the abstraction of solutes without water in the distal tubule, and if sodium salt cannot be reabsorbed at this site in adrenal insufficiency, it should be impossible to excrete a really dilute urine even in the total absence of ADH in this disorder. The site of action of aldosterone is not known and this is an important area for research.[18, 42] Studies with desoxycorticosterone glucoside and cortisone suggest that the former acts in the proximal, and perhaps the distal, tubule, whereas the latter seems to act at the distal site

alone.[58] It is of interest to mention that DOCA does not correct the water defect, whereas cortisone does.[10, 25]

Thus it is possible to ascribe a diminished ability to excrete water to some common denominator within the framework of the Smith hypothesis in a variety of disorders which appeared on the surface to share few resemblances. This is a successful response to the challenge and is an unequivocal demonstration of the usefulness of the hypothesis. Plausibility, however, cannot substitute for scientific proof, and one must be alert even to the minor inconsistencies between the theory and the observed data. These may represent important clues in the search for the missing pieces, or they may destroy a preconception and create a climate conducive to the development of a new concept. During the many discussions which the author was privileged to have with Dr. Peters certain general patterns of approach to scientific matters were recognized as part of Dr. Peters' genius. Important among these was the delight rather than the frustration with which he greeted data which appeared to be in conflict with each other or with a preconception (although his enthusiasm was somewhat dampened if the preconception was of his own design). He viewed these circumstances as opportunities to gain a further insight into the factors that condition some basic physiological response. This, in turn, reveals his greatest intellectual asset which was the capacity and the willingness to entertain a new idea. This is a worthy though difficult tradition to emulate.

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SERUM LIPIDS IN DIFFERENT PHASES OF CARBOHYDRATE METABOLISM‡

If the reader would integrate the intermediary metabolism of lipids with that of carbohydrates let him read, albeit in reverse direction, Dr. Peters' chapters on lipids and carbohydrate in Grollman's text,[82] the article on "The interrelations of foodstuffs in metabolism" in the 1951 Yale Journal of Biology and Medicine,[81] the chapter on lipids in the 1946 edition of *Quantitative clinical chemistry* by Peters and Van Slyke,[85] the 1941 Yale Journal article "A new frame for metabolism,"[80] and the chapter on lipoids in the 1931 edition of *Quantitative clinical chemistry*.[84] Certain landmarks, which Dr. Peters recognized before the era of isotope experiments, stand out: that fatty acids could be synthesized from carbohydrate or protein foodstuffs except for the essential, unsaturated fatty acids, that cholesterol could be synthesized endogenously and followed different routes in intermediary metabolism than triglycerides or phospholipids, that fat in adipose tissue was an invaluable source of fuel for energy metabolism.

The above source articles written throughout a span of 25 years give a forceful picture of interlocking relationships between the rôles of carbohydrate and fat in the physiological balance of storage and expenditure of bodily energy. From the stimulus of the above articles stemmed investigations on lipid fractions during recovery from diabetic acidosis and measurements of alimentary lipemia after a breakfast containing carbohydrate, proteins, and fat or after the same breakfast with supplementary ingestion of carbohydrate.

Diabetic acidosis affords a unique opportunity in which to examine rapid and extensive changes in serum lipid concentrations in man. Fatty acids of triglycerides increase excessively, sometimes to tenfold the normal maxi-

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‡ Editorial limitations have necessitated omission from the bibliography of many significant contributions. By inclusion of recent and review papers with extensive bibliographies appreciative recognition is extended to those investigators whose publications could not be cited individually.

The complete list of Dr. Peters' publications, which is in this issue, contains his articles on lipid metabolism which are not included in this bibliography.

mum, but during the first 24 hours of active treatment decrease to concentrations within or approaching the normal range.[5, 55, 65, 66, 65] Occasionally, serum cholesterol initially is as much as twice or more the concentration subsequently found to be characteristic for the individual. Phospholipids rise more than cholesterol but far less than triglycerides, if compared in a percentage relationship to their respective concentrations following convalescence. When the carbohydrate starvation and acidosis are clinically significant, the elevation of these three lipid fractions is far in excess of the degree of dehydration evaluated from changes in hematocrit or serum proteins.[55] The pattern of lipids given above has been found in usual instances of diabetic acidosis but hyperlipemias of extraordinary magnitude were observed in sporadic patients.[5] Characteristic abnormalities of the elevation of serum lipids in diabetic acidosis are the capricious distortion of interrelations in the lipid pattern and the load of triglycerides carried in the plasma.

Serum triglycerides of fatty acids thus attracted the focus of attention for the specific condition of diabetic acidosis in which carbohydrate metabolism is inseparable from that of lipids. In serum and in tissues the fraction of lipids which will be discussed involves these triglyceride fatty acids, calculated as the fatty acids not esterified with cholesterol or in phospholipids.[65, 66] In some of our earlier papers this triglyceride fraction was called "neutral fat" or "free fat." The latter name now is applied to that fraction of fatty acids, possibly an extremely active component in lipid turnover, which is unesterified in serum or plasma but seems to be bound to albumin and which by our calculations would be included in triglycerides.[18, 19, 20, 21, 66] By utilization of the term triglycerides the Committee on Lipid and Lipoprotein Nomenclature of the American Society for the Study of Arteriosclerosis has made no attempt to distinguish the nonesterified fatty acids or fatty acids of di- or monoglycerides from triglycerides.[16]

Triglyceride fatty acids represent the major component of fat in adipose tissue. Fat of adipose tissue is stored in pure form yielding about nine calories per gram in contrast to the energy potentials of carbohydrate or protein which, because they are held in aqueous deposits, yield only about one calory per gram of tissue.[61] Butter and the fat mechanically inseparable from the protein of meat are composed chiefly of triglycerides. Uncooked beef, veal, and lamb roasts, in addition to about 60 per cent of water, contain approximately equal per cents by weight of protein and fat. Thus for uncooked roast beef only 25 per cent of its caloric value is supplied by protein while 75 per cent would be derived from fat. This elementary fact is

often forgotten when a diet high in animal protein is needed to alleviate protein deficiency of a patient.

The vital rôle of triglycerides in meeting energy requirements of carnivorous animals has been emphasized because the size of this lipid fraction during transport in plasma would not indicate the magnitude of the rôle which the triglycerides exercise in total metabolism. An extremely rapid turnover of triglyceride fatty acids compared to the slower exchange of phospholipid and cholesterol has been demonstrated by isotope studies.[13, 34, 49, 66] The present concept is that triglyceride fatty acids are the form in which fat is available for fuel.

In Figure 1 the minimum, mean, and maximum fatty acids of triglycerides, phospholipids, and cholesterol esters in the sera of 100 normal men and women in the postabsorptive state are presented.[66] In some normal sera the triglyceride fatty acids were too low to be measured and accounted for only one-third, 3.1 to 6.1 mEq./L, of the total fatty acids for the sera of mean or maximum normal lipid content. Conversion of the maximum concentration to mM. gives the surprisingly small quantity of 2 mM./per L; if all of the fatty acid happened to be palmitic, the weight of palmitate would be only 161 mg. per 100 ml. of serum. As is obvious from the figure, the major compounds of fatty acids in sera are those combined in phospholipids and with cholesterol. In a given individual slight changes throughout the day or from day to day in the cholesterol and phospholipid fractions were exceeded by the variability in triglycerides. This variability in serum triglycerides in a given individual and the excessive elevation of triglycerides in the carbohydrate starvation of diabetic acidosis fit into the pattern of interrelationships of carbohydrate and lipid metabolism.

INTERMEDIARY METABOLISM OF LIPIDS

The biochemical reactions by which fat is metabolized have been reported with such extensive bibliographies that the following references are included only as a key to enter the literature on these subjects—12, 17, 25, 41, 51, 52, 53, 57, 58, 72, 74, 75, 78, 80, 86, 92. Acetyl Co-A is a necessary intermediate in the synthesis and degradation of fatty acids: any substance capable of forming acetyl Co-A can therefore serve as a substrate for fatty acid synthesis. Ability to synthesize fatty acids has now been demonstrated in many tissues including liver, mammary gland, intestinal mucosa, lung, heart, spleen, testis, and adipose tissues.[58] Synthesis and degradation of fatty acids from and to the two carbon acetate fragments occur through the activities of coenzyme A, and the energy-rich reactions of phosphorylation

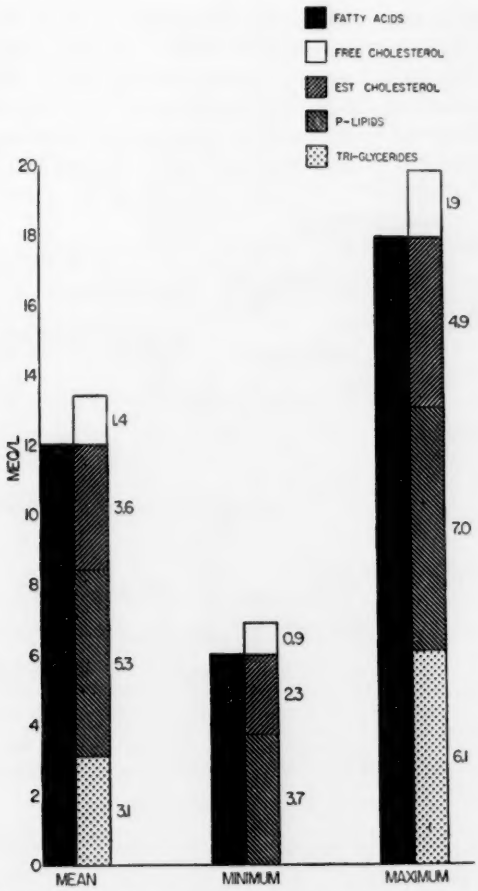

FIG. 1. Distribution of total fatty acids in serum lipid fractions. Cholesterol and lipid phosphorus are represented by the number of mEq./L of fatty acids with which they combine. Minimum and maximum total cholesterol (molecular wt. is 386) would be equivalent to the sums of the free and esterified cholesterol (2.3 + 0.9 mEq.) (386) (100 ml./1000 ml.) and (4.9 + 1.9 mEq.) (386) (100 ml./1000 ml.) or 123 to 262 mg. per cent of serum cholesterol.

Serum phospholipid composition is still being investigated but here 20 per cent of phospholipids is assumed to occur in sphingomyelin carrying 1 mEq. of fatty acids and 80 per cent in phospholipids carrying 2 mEq. of fatty acids per mM. of phosphorus. Phospholipid fatty acids would be calculated

$$\frac{(0.80 \times 2 + 0.20) \text{ lipid P in mg. per liter}}{31 \text{ (wt. of millimole of P)}}$$

Minimum and maximum mEq. of phospholipid fatty acids correspond with 6.4 to 12.0 mg. per cent of lipid phosphorus.

which interplay in energy exchange with the Krebs' cycle, Figure 2.* In the oxidation of fatty acids two mechanisms seem to predominate: either fatty acids are transported to the periphery for complete liberation of energy by degradation into two carbon fragments which are burned via the citric acid cycle, or in the liver fatty acids are broken down by activated coenzyme A into coenzyme A for re-utilization and into products yielding ketones.

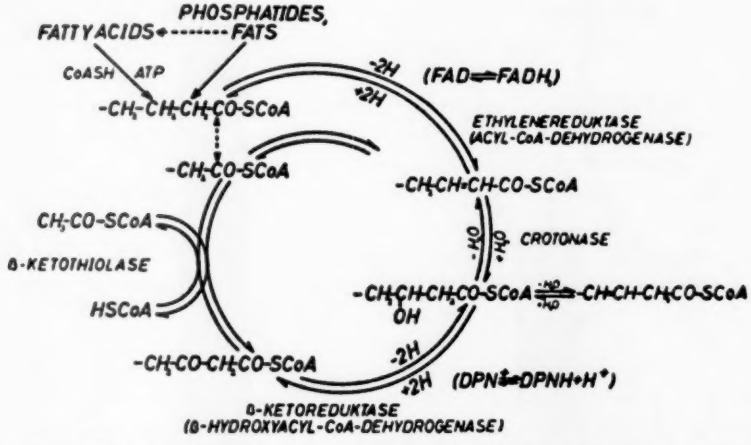

FIG. 2. Fatty acid cycle. Lynen has drawn a simplified diagram for the rôle of CoA in the metabolism of fat. This depicts the "(a) condensation of two molecules of acetyl-CoA to form acetoacetyl CoA and CoASH; (b) reduction of acetoacetyl CoA to B hydroxybutyryl CoA; (c) dehydration of B hydroxybutyryl CoA to crotonyl CoA; and (d) reduction of the crotonyl CoA to butyryl CoA." In this figure DPN (diphosphopyridine nucleotide) and FAD (flavin adenine dinucleotide) are included at steps of hydrogen exchange.[52, 53]

The latter after transfer to the periphery are burned by steps of the citric acid cycle. In tissues but not in liver "transferase" enzymes permit the utilization for energy of compounds, such as acetoacetate, which have been elaborated by the liver.[78]

Much published evidence, for which only a few articles are cited here, has confirmed not only the direct utilization for fuel of ketones but of fatty acids by tissues including heart and skeletal muscle, although the exact mechanism of transfer of fatty acids into muscle cells is not defined too pre-

* Since submission of this paper nomenclature for enzymes of fatty-acid metabolism has been suggested in the following reference: Beinert, H., Green, D. E., Hele, Priscilla, Hoffman-Ostenhof, O., Lynen, F., Ochoa, S., Popják, G., and Ruyssen: Nomenclature of enzymes of fatty-acid metabolism. Science, 1956, *124*, 614-616.

cisely.[28, 53, 76, 84, 87, 88] The major rôle which fat can assume in energy-producing reactions of the whole organism is indicated by the small percentage of oxygen utilized for combustion of glucose by fasting human subjects. Andres, Cader, and Zierler[7] studied the utilization of glucose by forearm muscle of postabsorptive human subjects and found that combustion of glucose accounted for only 7 per cent of the oxygen uptake, the remainder presumably being used for combustion of lipid and protein.

METABOLISM OF FAT IN RELATION TO THAT OF CARBOHYDRATE

The preponderance of metabolism of carbohydrate or fat is governed by the availability of these substances for fuel. Thus on a mixed diet, carbohydrate is liberally ingested during the day and burned as fuel. Excess carbohydrate is converted to glycogen and to fat for storage in the depots. During the overnight fast, fat is mobilized from the depots and by morning constitutes 60 per cent or more of the metabolic mixture.[61] From 8:00 a.m. to 10:00 p.m. in nonfasting individuals 60 grams of glucose are used by body muscle, compared to 30 grams used from 10:00 p.m. to 8:00 a.m.[28] As carbohydrate is further depleted with continued fasting, oxidation of both fatty acids and ketones increases to compensate for the energy requirements inadequately satisfied by carbohydrates. Separate reactions do not occur in a series. A continuously changing power supply involves carbohydrate ingestion, preferential combustion of carbohydrate and synthesis of fat during carbohydrate surplus; but when excess carbohydrate is spent, the newly formed fatty acids are immediately recalled for oxidation. Since the resultant RQ of these simultaneous reactions might be one, the RQ alone does not reveal the metabolic activity of fat in energy metabolism.[62] In a sense fat is the chief form in which fuel is burned by the body.

The velocity of carbohydrate combustion governs the *net* exchange of fat toward synthesis or combustion although certain adaptive phenomena and species differences alter the metabolic pathways for fat. Fat synthesis cannot generally occur without exogenous carbohydrate, but fat combustion in the absence of carbohydrate proceeds freely albeit with ketosis. This phenomenon was recognized by observation of severely diabetic, emaciated patients before the insulin era. That the nondiabetic animal could liberally synthesize fatty acids, with the exception of the essential, unsaturated compounds, was established by numerous investigations on the amount and composition of body fat of animals fed on "fat-free" diets or food of minimal fat content.[41, 64, 65] The extent of this phenomenon was revealed from experiments in which rats were fed on a carbohydrate diet containing glucose labelled with deuterium. Schoenheimer[70] demonstrated the dynamic, simul-

taneous breakdown and synthesis of fatty acids from the speed with which the label from glucose appeared in the fatty acids.

Following these early experiments, abundant evidence has accumulated that the synthesis of fat is dependent on the combustion of carbohydrate.[18] Haugaard and Stadie[37] found that the ability of liver to synthesize fatty acids from acetate was directly related to its glycogen content, and suggested that glucose not only provided the 2-carbon fragments, but also the energy for fatty acid synthesis. Since the rate of fatty acid synthesis varies with the supply of glycogen, and since liver tissue has a relatively high content of glycogen, investigations on liver tissue of the utilization of substrates between glycolysis and fat synthesis are complicated by the amounts of glycogen in the tissue. On the other hand, mammary tissue of lactating animals, which stores little glycogen, affords an excellent tissue to study the utilization for fat synthesis of such intermediates as glucose, fructose, pyruvate, or acetate.[39, 52, 67, 72, 90] Ruminants are less dependent than nonruminants on glucose for fat synthesis by mammary gland.[9] In this tissue the primary mechanism for fat synthesis depends on condensation of two carbon units rather than on elongation of the fatty acid chain. Glucose favors formation of triglycerides from the sodium salts of specific acids such as octanoic.[40]

While fatty acid synthesis requires the presence of glucose or products of the glycolytic cycle, these are not essential for fatty acid oxidation. The net exchange of fat in the direction of combustion is not facilitated but rather decreases when glucose or glycolytic products are available.[27, 88] Lossow and Chaikoff[50] followed the fate of labeled fatty acids injected as emulsions of triglycerides or as free fatty acids in rats with and without prefeeding with carbohydrate. The oxidation of all fatty acids of the even series from octanoic to palmitic was spared by carbohydrate, and the longer the chain, the more pronounced was the sparing. The intact labeled fatty acids were identified both in liver and in the whole carcass. Previous high carbohydrate diets, used in these experiments, may have intensified this effect.

The foregoing experiments demonstrate clearly that the active metabolism of glucose stimulates fatty acid synthesis and displaces fatty acid oxidation to a subsidiary position, while in the absence of carbohydrate fatty acid oxidation proceeds at an increased efficiency to meet the caloric demands and fatty acid synthesis fades into inactivity. It follows that all factors influencing carbohydrate metabolism might be expected to have an effect on fat metabolism opposite to their effect on carbohydrates. The effect of hormones in altering fat metabolism appears to be inseparable from their action on carbohydrate. It has been repeatedly demonstrated that insulin stimulates the formation of fat from glucose. The initial belief that insulin was necessary for a specific stage of fatty acid synthesis is not entirely dis-

proved[14] but has been replaced by the hypothesis that the action of insulin is secondary to its stimulating effect on carbohydrate metabolism.[75]

The inhibition of carbohydrate metabolism by the hormones of the anterior pituitary and the adrenal cortex is associated with decreased fatty acid synthesis and increased fat combustion.[14] In rat mammary slices the addition of cortisone *in vitro* inhibited lipogenesis.[8] Growth hormone has been reported to spare carbohydrate and protein *in vivo* by accelerating catabolism of fat provided dietary or carcass fat is available (Gurin in Najjar[56]). Allen, Medes, and Weinhouse[6] could not demonstrate an *in vitro* effect of growth hormone on fatty acid oxidation and enumerated the difficulties in using isolated tissues for such studies.

EFFECT OF GLUCOSE ON LIPIDS IN TRANSPORT

As a corollary to the fat-sparing, fat-synthesizing activities of glucose one might expect a surfeit of glucose to facilitate the deposition of fat in the depots, and the absence of glucose to favor fat mobilization. Since the plasma is the major vehicle for the transport of lipids, it might be anticipated that the relative predominance of mobilization or deposition of fat would be detected by changes in serum lipids; and indeed an increase in at least one and often all components of the serum lipids occurs when carbohydrate combustion is severely restricted. An example is the change occurring during fasting. Probably the earliest detectable change is a rise in the nonesterified fatty acids[19, 20] which occurs after an overnight fast, and which can be reversed by feeding 100 gms. of glucose. Gordon[20] gave evidence that the rise of nonesterified fatty acids is associated with mobilization of fat from the depots. There is considerable species variation in starvation. Man responds with a slight rise in serum cholesterol after five days of starvation, monkeys respond with a marked rise, and dogs display no consistent change in serum lipids and little ketosis.[43] The changes in man are prevented by the ingestion of as little as 100 gms. of carbohydrate a day.[43] In semi-starvation on a diet which is predominantly carbohydrate such changes as ketosis and elevation of lipids do not occur.[45] It was stressed by Peters that the ketosis and elevated serum cholesterol in starvation result from a specific lack of carbohydrate for some operative function rather than a caloric deficit.[45]

Factors which depress carbohydrate metabolism other than starvation may also cause elevations of serum lipids. An increase of triglycerides and other lipid fractions occurs in uncontrolled human diabetes, in glycogen storage disease, and in alloxan or pancreatectomy diabetes in experimental animals. Cortisone, growth hormone, and epinephrine favor fat mobilization and have been reported to cause lipemia in experimental animals.[3, 20]

Whether the effects of those substances on fat metabolism are primary or merely secondary to their inhibition of carbohydrate metabolism is a problem of no minor significance.

The hyperlipemia associated with impaired glucose metabolism is frequently attributed to intense mobilization of fat into the blood stream. That there might also be a delayed removal of fat from the blood stream is self-evident. Only sporadic experiments on this subject have appeared in the past. As early as 1918 Bang[10] showed in dogs that the feeding of oil alone produced a larger alimentary rise of plasma total fatty acids than when carbohydrate in the form of bread was fed with the same amount of oil. Rony and Ching[80] found that the rise in total fatty acids of plasma after a fat meal fed to dogs who had been fasted for 7 to 14 days could be abolished or decreased by the simultaneous administration of 1 gm. of glucose per pound of body weight, regardless of whether the glucose was administered orally, intravenously, or subcutaneously. Insulin had a similar effect, and epinephrine no effect. None of these substances had a demonstrable influence on the serum fatty acids of fasting dogs not given oil.

A more precise study of this phenomenon in human subjects was recently carried out in this laboratory,[8] using more detailed lipid analyses than were available to the above authors. The changes in serum lipids following a standard fat meal ingested after an overnight fast were compared with the changes occurring when extra glucose was administered orally with the fat meal. The fat meal consisted of 60 gms. of fat, 23 gms. of protein, and 42 gms. of carbohydrate. The glucose was given as lemonade in equal doses, one hour and again one-half hour before the breakfast. Occasionally, a third dose was given after breakfast. When the fat meal alone was given as a control to 12 normal individuals on 20 occasions, the only consistent change in the serum lipids was a rise in the emulsified triglyceride fraction from a mean fasting level of 3.1 mEq./L to a mean level of 6.6 mEq./L three hours after the fat meal.[4] The average increase was 3.5 mEq./L, with the minimum and maximum 1.5 and 5.5 mEq./L.* Lactescence occurred at triglyceride values between 3 and 11 mEq./L. In pathological lactescence of plasma in certain diseases the lipids appear in particulate form only when triglyceride concentrations exceed 15 to 20 mEq./L.[5] In such particles a large proportion of the plasma cholesterol and phospholipid is also found. If any cholesterol and phospholipid are present with the triglyceride particles of alimentary lipemia, their concentration is too small to be detected by the methods used. In striking contrast to the control series seven of eight sub-

* In previous experiments using a meal containing much more fat,[54] in addition to the rise in triglyceride fatty acids, lipid phosphorus increased slightly.

jects, each serving as his own control, responded to the administration of 120 gms. or less of extra glucose with a diminished or absent rise in triglycerides after the fat meal. Simultaneously, lactescence was less apparent in the serum. From these findings,[4] it was concluded that active carbohydrate metabolism facilitated the escape of particulate lipids from plasma following a fat meal but that interpretation of individual differences might disentangle the metabolic processes involved in this phenomenon. Any substance capable of stimulating carbohydrate metabolism might have the same effect without the necessity of giving carbohydrate itself, and substances inhibiting carbohydrate combustion might have an opposite influence. This prediction has been confirmed thus far with two substances—glucagon, which diminishes, and epinephrine, which if anything increases alimentary lipemia.[4] Other investigators found more unabsorbed fat in intestinal chyme obtained near Treitz' ligament during the two hours after ingestion of 45 gms. of vegetable fat alone than when bread, jelly, sugar, and coffee were consumed with the same amount of fat,[86] verifying our opinion that the effect of glucose is not a local action on the gastrointestinal tract.

The mechanism by which glucose reduced the rise in triglyceride fatty acids after the standard breakfast is unknown. Most workers in this field have directed their attention to alimentary lipemia after dietary fat without due consideration of the status of carbohydrate metabolism. The two most common metabolic processes for reduction of any fuel such as plasma triglycerides are oxidative combustion or deposition and storage. That fat is preferentially spared in carbohydrate surplus has been emphasized, leaving the alternate explanation that glucose facilitates removal of triglycerides for deposition and storage. Spitzer[78] in agreement with others has called attention to the capacity of the tissues of the dog to remove fat from the blood when he observed less lactescence in venous than in arterial blood of the leg of the hepatectomized dog previously given olive oil. Bergström and associates,[13] from quantitative comparisons of specific activities in fatty acids of lymph and plasma triglycerides, assumed that special properties of emulsified fat from thoracic duct lymph promoted its absorption from systemic blood. Although direct entry of emulsified lipids into adipose tissue and utilization by muscle cells of triglyceride fatty acids are two possible metabolic pathways for reduction of plasma lipids (Chaikoff and Brown in Greenberg[30]), the liver may play a major preparatory rôle after fat in the chyle enters the blood stream. To the liver is generally attributed the rôle of lipoprotein formation from circulating triglyceride particles removed for incorporation into lipoproteins; the latter are then released to the plasma for transport to the periphery where the fatty acids from triglycerides are used for fuel. Waddell *et al.*[85] demonstrated in rats that the removal of intra-

venously injected fat emulsions was considerably retarded by hepatectomy. Friedman *et al.*[54] attributed to the Kupffer cells of the liver removal of cholesterol-containing chylomicrons. Spitzer[78] reported that the turbidity of serum in dogs fed cod liver oil actually increased following functional hepatectomy. The liver has an important rôle in the metabolism of mobilized as well as administered fat. A transfer of depot fat to the liver occurs under the stimulus of growth hormone[79] and in female rats, following starvation.[91] If removal of fat from the liver is blocked by administering ethionine, a substance which is thought to interfere with protein formation, striking hypolipemia and fatty liver develop.[25] In all the studies quoted above, carbohydrate metabolism may have been impaired by starvation, by growth hormone, by hepatectomy, or by the administration of fat with little or no carbohydrate. If the absence of exogenous glucose favors mobilization and combustion of fat, the foregoing studies elucidate the fate only of fat destined to be burned, and leave unknown its disposition when an ample supply of carbohydrate favors its storage. It seems entirely possible that carbohydrate plenty activates enzyme systems of the depots to remove circulating fat directly, and in the absence of sufficient carbohydrate enzymatic functions of the depots to remove circulating fat are temporarily inactive. Circulating fat would then persist in the plasma until removed by the liver and other organs.

ADIPOSE TISSUE

Shapiro and Wertheimer[71] cite recent vivid reports of the rapid biochemical activity of adipose tissue. The reactions, which include synthesis of glycogen and fat, vary not only from animal to animal but with the anatomical locations and mechanical function of the fat. Oxygen consumption does not differ, but respiratory quotients are higher if tissues from animals fasted for 24 hours are compared with fat depleted tissues. Fat synthesis persists into the fasting state in adipose tissue at a time when it is greatly curtailed in liver.[11] Feller,[28] using male mice fed ad libitum, found greater synthesis of fatty acids in adipose tissue than in liver, as did Hausberger and Milstein,[46] while Itzhake and Wertheimer (cited by Shapiro and Wertheimer[71]), who worked with normally fed rats, reported that respiratory activity of adipose tissue was half that of liver. In each instance the large amounts of fat and water in adipose tissue and liver necessitate the conversion of data to nitrogen content of fat free dry weight. Feller[28] found different activities dependent on the position of the labelled carbon of acetate. Stern and Shapiro[77] incubated for four hours adipose tissue in serum or serum enriched with fat and noted transfer from serum to adipose

tissue of 1.3 to 4.2 mEq. of fatty acids. *In vivo* preferential selection by adipose tissue of specific fatty acids occurs, but after feeding fat of atypical nature the character of the fat in depots approaches the composition of dietary fat (Chaikoff and Brown in Greenberg[30]).[65, 72] The presence in adipose tissue of a lipoprotein lipase having clearing activities[47] further implicates this tissue in the clearing of alimentary lipemia. Adipose tissue may also play a vital rôle such as desaturation[71] in preparing ingested fat for subsequent use as fuel, in addition to providing storage space for fat. From this mass of diverse experimental results it might be conceded that adipose tissue derives triglycerides not only by direct deposition from the blood stream, but also by synthesis from carbohydrate.

The effect of glucose on alimentary lipemia might be mediated through alterations in cellular permeability to triglycerides of fatty acids. Insulin, which is probably secreted in response to administered glucose, may increase cellular permeability to certain sugars,[74, 75] but it is not known whether this pertains to other substances. Adlersberg *et al.*[1] suggested that corticosteroids have the opposite effect of decreasing tissue permeability, a finding of particular interest in view of the antagonistic action of insulin and cortisone on carbohydrate metabolism.

A comparison of the effect of glucose with that of heparin in reducing alimentary lipemia is inevitable. *In vivo* visual clearing of lipemic serum following heparin injection or *in vitro* clearing after incubation with serum from heparinized animals[82] is accompanied by a rise of albumin-bound nonesterified fatty acids released by hydrolysis of triglycerides.[61, 66] *In vivo* studies indicate not only a physical change but also an escape of fat from the blood stream following heparin.[78] Jeffries[43] claimed no definite relationship between clearing activity caused by heparin and clearing activity which he observed in plasma of rats after feeding fat. Intravascular hydrolysis of triglycerides and subsequent removal of the liberated fatty acids may be a part of the physiological mechanism for clearing alimentary lipemia. By removal of the end product, nonesterified fatty acids, further hydrolysis of triglycerides might be promoted. Diminution of nonesterified fatty acids has been reported after feeding glucose.[19, 39] On the other hand, glucose might, consistent with its sparing action on fatty acids,[66] inhibit the hydrolysis of triglycerides, facilitating rather their removal *in toto* for storage. Bergström and Borgström[12] have just reviewed about 40 investigations on the clearing factor and have discussed the rôle of numerous substances, of lipoproteins, of albumin and calcium as acceptors of nonesterified fatty acids, of glycogen, of alimentary lipemic plasma in activity of the clearing factor. Their conclusions, strangely appropriate to the observations in our alimentary lipemia experiments, are that *in vivo* lipemic blood is cleared by passage through the

capillaries of such tissues as heart and adipose tissues, and that overflow from these sites of clearing factor accounts for its presence in plasma. Much evidence does suggest that such overflow occurs following increased activity of fat storage.

SPECIES DIFFERENCES

Wide species variations in carbohydrate and fat metabolism have been emphasized.[69, 72] These differences are briefly mentioned again because they are important in selection of experimental animals to study alimentary lipemia. Animals such as dogs, which normally eat little carbohydrate, respond to starvation with little ketosis or change in serum lipids.[43] Rony[69] noted that lipemia rarely increased in dogs after a fat meal after a 24-hour fast. He noted that a preliminary fast of 7 to 14 days was necessary to promote a consistent alimentary lipemia. Conversely, the carbohydrate-dependent rabbit, which is remarkably sensitive to carbohydrate withdrawal, develops lipemia with facility not only after fat feeding but also following almost every conceivable stimulus. It appears that after an overnight fast animals which are able to conserve their carbohydrate on fasting demonstrate less alimentary lipemia than those which become rapidly depleted of glycogen.

ADAPTATION

The foregoing data would suggest that in view of the reciprocal relationship of fat to carbohydrate metabolism, metabolic pathways of fat are governed by dietary supply of carbohydrate. Many observations, however, indicate that this relationship persists only in animals receiving ample dietary carbohydrate. Animals maintained for a long period of time on a carbohydrate-limited diet develop adaptive changes which may relieve fat metabolism of its dependency on carbohydrate. This phenomenon has been studied by comparing the response to starvation of animals previously adapted to a high carbohydrate diet with those adapted to a high fat, low carbohydrate diet. As outlined above, the carbohydrate-adapted rat, upon withdrawal of carbohydrate, rapidly becomes ketotic, depleted of liver glycogen, and loses the ability to synthesize fatty acids. Such an animal continues to burn his available stored glucose until severe carbohydrate depletion results. However, these changes are minimized by previous high fat rations for several weeks. Whitney *et al.*[89, 90] summarized a number of metabolic changes which they had reported previously as occurring in fat-adapted animals. On food withdrawal, these animals develop less ketosis and are less sensitive to insulin. These responses correlate with *in vitro*

studies of liver slices, in which glycogen stores were guarded during fasting, and which, on incubation with glucose, utilized glucose sparingly with a corresponding reduction in glycogenesis, carbon dioxide production, and lipogenesis. Rat diaphragm oxidized more added palmitic acid when obtained from a fat-adapted than from a carbohydrate-adapted animal.[88] The fat-adapted animal is thus able to burn fatty acids before carbohydrate depletion occurs. Such an animal guards his carbohydrate supply by curtailing the metabolic activity of glucose.

In human subjects Sweeney[81] showed that two days of starvation or of a high fat, carbohydrate-free diet had similar effects in impairing carbohydrate tolerance. Ketosis and hypoglycemia diminished when human volunteers have starved for periods of 2½ days[82] at six-week intervals. This adaptive response of long duration has also been reported for obese individuals maintained on diets containing 1 gm. of protein and 0.6 gms. of carbohydrate per kg. ideal weight, but supplying only 400-600 calories per day.[21] During the first few weeks, not only fat but stored carbohydrate and nitrogen were lost. Later nitrogen equilibrium was established, and only body fats were burned. The adaptive changes on a high fat diet may actually be a result of the low carbohydrate in these diets. In the regulation of dietary fat intake the fact should not be neglected that a low fat diet is usually a high carbohydrate diet, and that the interaction of carbohydrate and fat metabolism is subject to adaptive changes. A portion of ingested carbohydrate is normally converted to fat before being burned and this proportion may be increased on a low fat, high carbohydrate diet thus to some extent negating the intent of the low fat diet.

The adaptive response evoked by a low carbohydrate, high fat diet has clinical application to the problem of functional hypoglycemia, and probably accounts for the success of high fat, high protein, low carbohydrate diets used to treat this condition. Such a diet has also been used in the treatment of obesity[89] on the premise that the absence of carbohydrate favors fatty acid oxidation and thus the burning of endogenous fat. With adaptive changes carbohydrate sparing should prevent hypoglycemic hunger attacks and subsequent gorging so common in the obese patient on a restricted diet. The ability of a patient to gain weight and retain nitrogen on a prolonged (5 months) diet consisting solely of fat and protein[46] demonstrates that, with adaptation, fat storage *can* occur in the absence of exogenous carbohydrate. A part of the adaptive phenomenon appears to be the ability to revise certain functions normally dependent on exogenous carbohydrate when the source of the latter is limited.

Adaptive changes occurring during long periods of high-fat feeding or successive starvation are not understood. They may represent changes in

activity of enzyme systems or in hormonal control. There is some support for an "adipokinetic" hormone of the anterior pituitary which mobilizes fat and increases fat combustion. Li[48] discussed the fat-burning, protein-sparing activities of the pituitary as properties of growth hormone. Whatever the mechanisms, the end result favors the conservation of endogenous carbohydrate by the preferential burning of fat for energy. The fat-adapted animal, having already substituted fat for carbohydrate as the major fuel, develops less severe carbohydrate depletion and subsequent ketosis during starvation.

The significance of carbohydrate in human diets seems to have been pushed aside in many discussions of human disease in which abnormalities of lipid metabolism occur. Not only does carbohydrate play a major rôle in governing combustion and synthesis of fat in the framework of metabolism, but the relative quantities of carbohydrate given for energy supply influence interrelated protein and lipid metabolic processes essential to the normal catabolic pathways followed by fat. The differences in specific activities of fat after giving C-14 stearic or palmitic triglycerides[18] afford only one example of the many differences in combustion and storage of diverse forms of fat.[20, 44, 73] The influence of protein and amino acid supply on plasma lipids is mediated through their rôle in phospholipid and lipoprotein formation by the liver. Unsaturated, essential fatty acids play a distinct but not completely defined rôle in formation and transport of phospholipids and esterified cholesterol.[2, 18, 41]

The studies on adaptation demonstrate singularly well the futility of studying a single fuel without due consideration of the status of other foodstuffs and the metabolic and nutritional state of the animal. Whether the effects of many of the factors that alter the metabolism of fat are secondary to their inhibition or facilitation of carbohydrate metabolism is a problem of major significance in the investigation of energy exchange.

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THE METABOLISM OF FRUCTOSE IN MAN§¶

The metabolism of fructose has been studied with varying intensity and enthusiasm since Kulz[12] in 1874 first reported that diabetic patients were able to utilize fructose given orally better than other sugars. Although Naunyn[20] confirmed these results, he maintained that tolerance for fructose was eventually lost, and Joslin,[11] as late as 1946, stated that in his experiments "at first the fructose was well-utilized, but later hyperglycemia and glycosuria usually followed." Interestingly enough, however, the same authors found that a diet containing Jerusalem artichokes, which on digestion yield fructose, could be taken for a period of at least three years without the subject losing his ability to utilize it more efficiently as compared with an equal amount of starch. The advent of insulin eliminated much of the clinical interest in fructose as an adjunct in the treatment of diabetes mellitus in man.

Despite the inconsistent and often contradictory results reported in patients, it seemed clear in experimental diabetes in animals that fructose was handled differently from glucose and did not require the intervention of insulin. Minkowski[19] in 1893 observed that fructose, in contrast to glucose, led to the formation of glycogen in the liver when given to depancreatized dogs. In 1929 Cori[2] studied the utilization in muscle of known amounts of intravenously injected fructose and glucose in eviscerated, depancreatized rats. By subsequently analyzing the entire carcasses for free sugar, he found that 39 per cent of the fructose and only 10 per cent of the glucose had disappeared in one hour. The injection of insulin resulted in a marked increase in the rate of disappearance of glucose but had no effect on the metabolism of fructose. A possible explanation for these findings lies in the

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demonstration in 1950 by Slein and Cori[28] that separate enzymes existed in muscle for the phosphorylation of fructose and glucose. Insulin apparently acts only on the glucokinase. In liver, also, a separate enzyme has been identified for the phosphorylation of fructose. In 1951 Chernick and Chaikoff,[8] using C^{14}-labelled fructose, found no significant difference in the capacities of normal and diabetic rat livers to oxidize fructose to CO_2.

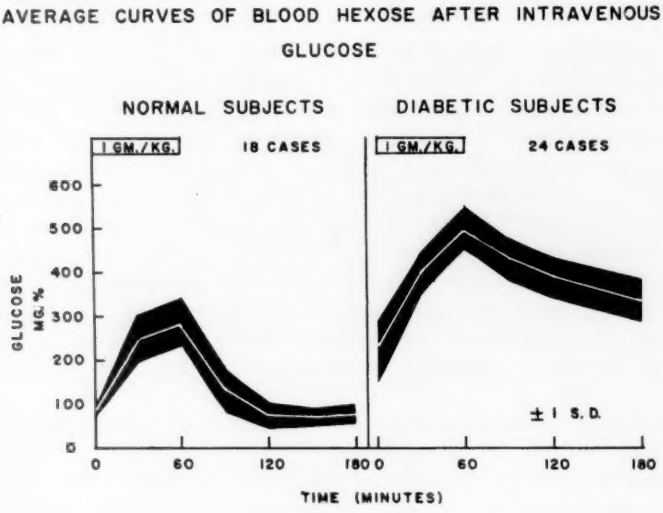

FIG. 1.

With these definitive results in the experimental animal it seemed logical to re-investigate the metabolism of fructose in man. The increase in knowledge of intermediary metabolism in the last two decades, the introduction of isotopes for clinical use, the development of accurate chemical methods for determining glucose and fructose separately in body fluids, and the availability of purified fructose for oral and intravenous use* suggested to us that the time was opportune. Beginning in 1951[4, 17] an intensive and systematic study has been pursued in our laboratories. Since the oral administration of fructose could not be expected to demonstrate quantitatively the full effects of fructose, because of the conversion of at least a portion to glucose by both intestinal mucosa and liver,[1, 30] attention was directed mainly

* The fructose in the studies to be reported was generously supplied by Dr. Warren Cox, Jr., and Dr. Robert Little of Mead Johnson and Company.

toward the metabolism of intravenously administered fructose. In most of the experiments fructose in amounts of 1.0 gm. per Kg. was given intravenously in a 10 per cent solution at a constant rate over a period of 60 minutes. Where possible, the results obtained were compared in the same individual with glucose administered in a similar way. In the diabetic subjects all tests were performed in the absence of any detectable insulin effects,

AVERAGE CURVES OF BLOOD HEXOSE AFTER INTRAVENOUS FRUCTOSE

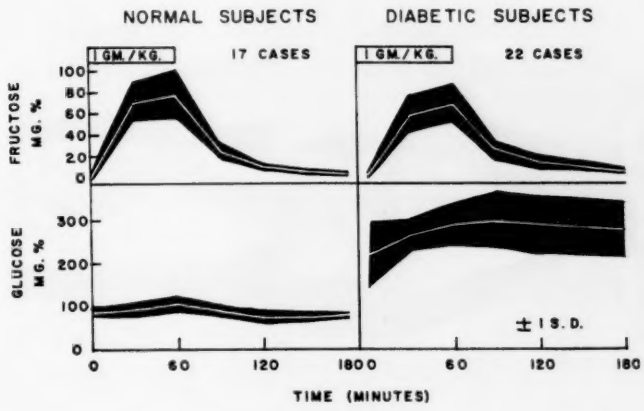

FIG. 2.

since the patients had previously been controlled on regular or crystalline insulin, the last dose having been given at least 14 hours before the test. In addition, in certain instances the results in the diabetic subject were compared with those obtained in the normal person. Fructose and glucose levels were determined separately in both blood and urine. Changes in blood pyruvic acid and serum inorganic phosphorus levels were determined in certain cases as additional indices of carbohydrate metabolism (see reference 17 for details of methods).

COMPARISON OF THE METABOLISM OF INTRAVENOUS GLUCOSE AND FRUCTOSE IN NORMAL SUBJECTS[17]

In 18 normal individuals after the infusion of 1.0 gm./kg./hr. of glucose the average maximum rise in blood glucose above the initial level was 215 mg./100 ml. (Fig. 1). With fructose, however, the average rise in

blood fructose above the control value in 17 of these same cases was 77 mg./100 ml., less than one-half that obtained with glucose given in comparable amounts and at the same rate. A slight rise in blood glucose following fructose administration was noted in some instances, the average maximum rise being 18 mg./100 ml., occurring at one hour (Fig. 2). The excretion of hexose in the urine during the period of the infusions and the

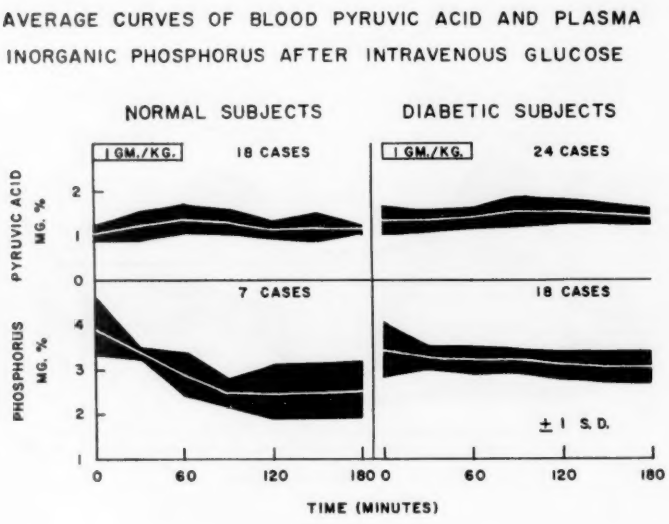

FIG. 3.

subsequent two hours was less than 10 per cent of the amount administered, 9.3 per cent after glucose and 5.3 per cent after fructose. That any fructose is excreted is explained by the fact that fructose continues to be excreted even at blood levels as low as 4 mg./100 ml., but the smaller rise in blood level minimizes the amount lost in the urine.

Pyruvic acid levels in the blood rose after glucose infusion on the average of 28 per cent, or 0.30 gm./100 ml. above the control level, the peak occurring at 60 minutes (Fig. 3). This rise, although small in absolute terms, is significant, the associated probability being of the order of less than one-half of 1 per cent that so great a difference should arise by chance in random sampling. In contrast, the pyruvate rise after fructose was strikingly higher, averaging 110 per cent, or 1.22 mg./100 ml. above the level at the start (Fig. 4). Serum inorganic phosphorus fell after either glucose or fructose,

but the magnitude and rate of change differed slightly. With glucose the phosphorus fell progressively 1.39 mg./100 ml., or 31 per cent below the initial level over a period of 90 minutes and then leveled off. After fructose the phosphorus fall was more rapid, reaching its lowest point at 30 minutes and then leveling off at a slightly higher value after 90 minutes (compare Figs. 3 and 4, normal subjects).

AVERAGE CURVES OF BLOOD PYRUVIC ACID AND PLASMA INORGANIC PHOSPHORUS AFTER INTRAVENOUS FRUCTOSE

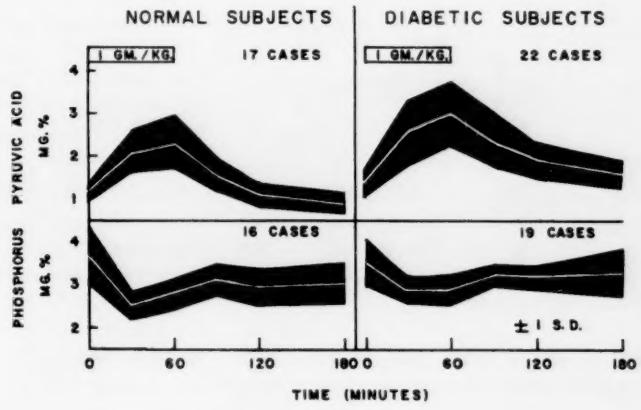

FIG. 4.

These results in the normal subject permit the following conclusions: (i) Fructose is removed from the blood stream more than twice as rapidly as glucose during the period of infusion; (ii) the rise in blood glucose following fructose administration indicates that a fraction of the fructose must have been converted to glucose; (iii) the rise in pyruvate can be interpreted as an indication that both glucose and fructose are metabolized by the way of the Embden-Meyerhof series of reactions. The much greater rise after fructose is probably a reflection of its entrance into the metabolic scheme closer to pyruvic acid. Hers[10] has shown in rats that fructose is transformed into liver and muscle glycogen by splitting to the triose state and entering the glycolytic scheme just below fructose-1, 6-diphosphate. The *in vivo* studies in man are consistent with this hypothesis. If the fall in serum inorganic phosphorus reflects the process of phosphorylation in

the metabolism of carbohydrate, the more rapid decrease in phosphate after fructose administration is an indication of the more rapid metabolism or utilization of this hexose.

The above changes in the concentrations of hexose, pyruvate, and phosphate after intravenously administered glucose and fructose are compared in Table 1. Statistical measures of the variability are included.

TABLE 1. CHANGES IN GLUCOSE, FRUCTOSE, PYRUVATE, AND PHOSPHATE IN NORMAL SUBJECTS AFTER THE INTRAVENOUS INFUSION OF 1.0 GM./KG. IN ONE HOUR OF (A) GLUCOSE (B) FRUCTOSE

Substance measured in blood	*Time minutes from start*	*(A) After glucose*		*(B) After fructose*	
		Δ mg./100 ml.	*Per cent change*	*Δ mg./100 ml.*	*Per cent change*
Fructose	60			+77.2 ±23.2*	
Glucose	60	+214.7 ±52.2*		+18.5 ±18.4	
Pyruvate	60	+0.30± 0.34	+28	+1.22± 0.62	+110
Phosphate	30	—0.56± 0.11	—14.7	—0.93± 0.34	—25.6
"	90	—1.39± 0.31	—36.5	—0.51± 0.37	—14.1

* ± 1 Standard deviation.

COMPARISON OF THE METABOLISM OF INTRAVENOUS FRUCTOSE AND GLUCOSE IN DIABETIC SUBJECTS (Figs. 1, 2, 3, 4)

Intravenous glucose and fructose tolerance tests were performed one or more times in each of 15 patients with diabetes mellitus. Two other subjects were given glucose and one fructose, making a total of 24 glucose and 22 fructose tolerance experiments. The diabetes in general was of moderate to severe intensity, with an average fasting blood sugar of 220 mg./100 ml. In only two patients was the fasting blood sugar below 140 mg./100 ml., and these were the only two that did not require insulin for control. When both tests were performed in the same individual, they were done at one- to three-day intervals, and the fasting blood sugars were in the same range on each occasion.

Following intravenous glucose (Fig. 1) the changes in blood glucose were characteristic of diabetes mellitus, with marked hyperglycemia and a delayed fall. In marked contrast, the blood disappearance curve for fructose was in every case essentially identical with that found in the normal group (Fig. 2). Corresponding to these similar blood levels, the amount of fructose excreted in the urine of the diabetic patients during the period of

the infusion and the subsequent two hours was essentially the same (average 3.2 gms., range 0.9-5.7 gms.) as in the normal (average 3.6 gms., range 1.2-5.9 gms.).

The diabetic subject differed from the normal in that there was a more marked rise in blood glucose following fructose, a rise roughly proportional to the severity of the diabetes (Fig. 5). In every instance where direct comparisons were made, this blood glucose elevation was always less than after glucose injection. This is shown in Figures 1 and 2, where the changes in blood glucose in diabetic subjects are averaged. Direct quantitative comparison is shown in Table 2, where the increment in glucose and total hexose above the control level is shown at various intervals following this infusion. Since the blood sugar curves are not as high after fructose infusion, the excretion of hexose (glucose + fructose) is correspondingly less. In each of 12 subjects whose initial blood glucose levels were similar at the time of the glucose and fructose experiments, significantly less hexose was found in the urine after fructose. As pointed out above, the excretion of fructose was the same as that found in normal subjects, but the amount of glucose lost varied with the severity of the diabetes. Approximately 20 per cent more of the total amount infused was retained or "utilized" when fructose was administered (Table 3). It is interesting that this advantage is independent of the severity of the diabetes. In Table 3 the subjects are arranged according to increasing amounts of glucose lost in the urine in the three-hour period. In the first six subjects who excreted an average of 30 per cent (range 20.7 to 36.9 per cent) of the infused glucose, 19.9 per cent more hexose was "utilized" after fructose. In the other six patients whose diabetes was of such severity that 58.6 per cent (range 39.1 to 89.6 per cent) of the infused glucose was excreted, the "advantage" of fructose was still 20.7 per cent. Furthermore, in one case of experimentally produced diabetic acidosis, the subject "utilized" 178 grams of fructose or 21 per cent of the 850 grams administered intravenously during the phase when insulin

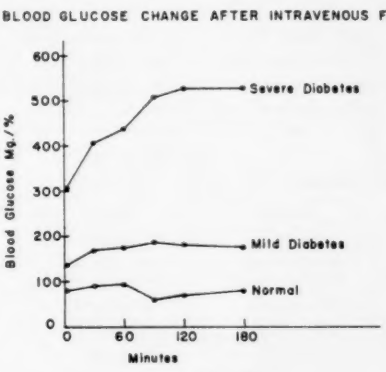

FIG. 5.

was withheld. Less than 4 per cent of the 850 grams of fructose was excreted as such in the urine. In three other similar experiments in the same volunteer, when fructose was not administered, there was a quantitative excretion of all available glucose during the withdrawal phase. Thus, even the "complete" diabetic individual obtains the same "advantage" from intravenous fructose as the less severe case.

Another striking difference in the metabolism of glucose and fructose in the diabetic subject was shown by the changes in blood pyruvic acid (Figs.

TABLE 2. INCREASE IN BLOOD GLUCOSE AND TOTAL HEXOSE FOLLOWING FRUCTOSE INFUSION COMPARED TO INCREASE IN BLOOD GLUCOSE FOLLOWING GLUCOSE INFUSION IN DIABETIC SUBJECTS
AMOUNT INFUSED: 1.0 GM./KG. IN ONE HOUR

No. of experiments	*Rise after fructose* 22			*Rise after glucose* 24
Time minutes	*Blood fructose mg./100 ml.*	*Blood glucose mg./100 ml.*	*Total blood hexose mg./100 ml.*	*Blood glucose mg./100 ml.*
30	58.3±16.9*	43.1±35.4*	101.4	176.7±48.5*
60	67.9±17.6	69.0±49.6	136.9	280.1±47.5
90	23.0± 8.5	82.1±66.0	105.1	207.0±49.2
120	12.3± 5.7	68.6±64.6	80.9	166.8±46.0
180	3.7± 2.8	56.8±64.0	60.5	116.1±47.4

* ± 1 Standard deviation.
Initial blood glucose values 221.1±76.6 and 220.1±69.1 mg./100 ml., respectively.

3 and 4). After glucose a significant rise in pyruvate did not occur until 90 minutes and this rise was sustained even at 180 minutes. In 18 normal subjects the maximum rise occurred at 60 minutes or earlier in 10 instances, compared with a similar early rise in only 1 out of 24 experiments in diabetic subjects. Furthermore, in 7 of these 24 diabetic patients no rise at all was found. In contrast, the rise in pyruvate after fructose administration in the diabetic state occurred just as promptly as in the normal subject. The magnitude of this increase was at least as great as in the nondiabetic, and was significantly higher at from 90 to 180 minutes.

In diabetic subjects the response of serum inorganic phosphorus to the administration of intravenous glucose and fructose is also markedly different (Figs. 3 and 4). After glucose, only a slight fall, statistically not significant, occurs in the first 90 minutes. After fructose, however, there is the

TABLE 3. COMPARISON OF UTILIZATION OF FRUCTOSE AND GLUCOSE IN DIABETIC SUBJECTS

Subject no.	*Hexose* infused*	*% of amount infused excreted as hexose*		*Additional amount of hexose "utilized" after fructose % of amount infused*
		After glucose†	*After fructose‡*	
1	Glucose	20.7		
	Fructose		6.0	14.7
2	Glucose	23.4		
	Fructose		5.6	17.8
3	Glucose	28.7		
	Fructose		5.9	22.8
4	Glucose	32.6		
	Fructose		15.2	17.4
5	Glucose	35.0		
	Fructose		15.7	19.3
6	Glucose	36.9		
	Fructose		9.3	27.6
			Average of Subjects No. 1-6:	19.9%
7	Glucose	39.1		
	Fructose		6.4	32.7
8	Glucose	43.1		
	Fructose		17.6	25.5
9	Glucose	54.0		
	Fructose		37.4	16.6
10	Glucose	55.6		
	Fructose		36.0	19.6
11	Glucose	70.0		
	Fructose		59.3	10.7
12	Glucose	89.6		
	Fructose		70.6	19.0
			Average of Subjects No. 7-12:	20.7%

* 1.0 gm./kg. infused intravenously in one hour.
† Glucose in urine in first three hours.
‡ Glucose and fructose in urine in first three hours.

same rapid fall, somewhat less in magnitude compared to the normal, in the first 30 minutes, with a rise after 90 minutes.

The results in the diabetic subject in the absence of insulin effect indicate that there is very little impairment in the metabolism of fructose as shown by: (i) The essentially similar rate of removal from the blood stream as in the normal person; (ii) no reduction in the height of pyruvate rise, and

TABLE 4. CHANGES IN GLUCOSE, FRUCTOSE, PYRUVATE, AND PHOSPHATE IN DIABETIC SUBJECTS AFTER THE INTRAVENOUS INFUSION OF 1.0 GM./KG. IN ONE HOUR OF (A) GLUCOSE (B) FRUCTOSE

Substance measured in blood	*Time minutes from start*	*(A) After glucose*		*(B) After fructose*	
		mg./100 ml.	*Per cent change*	*mg./100 ml.*	*Per cent change*
Fructose	60			+67.9 ±17.6*	
	180			+3.7 ± 2.8	
Glucose	60	+280.1 ±47.5*		+69.0 ±49.6	
	180	+116.1 ±47.4		+56.8 ±64.0	
Pyruvate	30	+0.001± 0.25	0.0	+1.17± 0.77	+85.4
	60	+0.06 ± 0.25	+4.8	+1.62± 0.76	+118.2
	90	+0.23 ± 0.33	+18.3	+1.04± 0.65	+75.9
	120	+0.25 ± 0.30	+19.9	+0.55± 0.43	+40.1
Phosphate	30	—0.17 ± 0.27	—4.9	—0.63± 0.31	—18.0
	60	—0.23 ± 0.31	—6.7	—0.61± 0.35	—17.4
	90	—0.26 ± 0.27	—7.5	—0.27± 0.27	—7.1

* ± 1 Standard deviation.

(iii) rapid fall in serum inorganic phosphate. In contrast, in parallel circumstances, glucose disappearance from the blood is markedly delayed, the rise in pyruvate is either abolished or delayed in appearance, and phosphate fall is not significant.

These changes in the diabetic subject after the intravenous administration of glucose and fructose are summarized in Table 4. The values should be compared with those found in normal subjects shown in Table 1.

ORAL FRUCTOSE VERSUS INTRAVENOUS FRUCTOSE IN THE DIABETIC SUBJECT

The effects of fructose given by mouth were compared in three individuals with the same amount given intravenously. The fructose was dissolved in water and ingested as a 10 per cent solution within a few minutes at the

beginning of an experiment, while the same amount was given intravenously as a 10 per cent solution in 60 minutes on another occasion. In each instance after oral administration the blood fructose level was much lower than that found after intravenous injection, while the elevation of blood glucose was more than twice as great and remained higher for a longer period of time. Part of the fructose, therefore, must have been converted to glucose in its passage through the intestinal mucosa and the liver. A typical experiment is shown in Table 5. The augmented excretion of glucose after oral fructose emphasizes the advantages of the intravenous route.

In order to determine more directly the amount of conversion of fructose to glucose by the intestinal mucosa a patient with cirrhosis of the liver and

TABLE 5. COMPARISON OF EXCRETION OF HEXOSE IN A DIABETIC SUBJECT AFTER (A) ORAL AND (B) INTRAVENOUS FRUCTOSE

				Amount excreted in 3 hours		
Subject	*Amount given gm.*	*Route of administration*	*Fasting blood glucose*	*Glucose gm.*	*Fructose gm.*	*Total hexose gm.*
S.	100	Oral	279	28.6	0.9	29.5
	100	Intravenous	283	15.7	5.2	20.9

portal obstruction with large superficial abdominal anastomotic veins was given 50 grams of fructose by mouth. Femoral artery blood and portal anastomotic vein blood samples were collected simultaneously in 30 and 60 minutes and analyzed for fructose and glucose. The results in this experiment are shown in Table 6. If the assumption is made that the *utilization* of hexose by the intestinal wall is negligible, then the ratio of the increase in portal anastomotic vein (PAV) blood glucose to total hexose (fructose + glucose) will be an approximate measure of the conversion of fructose to glucose during the passage through the wall. Thus, in the first sample obtained at 30 minutes, the concentration of glucose in PAV blood was 5 mg./100 ml. higher than in the arterial blood reaching the intestine. Since this could only have been derived by conversion from fructose (no other food being given), and the total hexose absorbed from the intestine was 28 mg./100 ml. (23 + 5) the fraction of fructose converted to glucose during the transfer through the wall was 5/28 or approximately 18 per cent. The results obtained in the second sample were in agreement with the first. Thus, about one-sixth of the fructose in this experiment was converted to glucose during absorption. Consequently, there is less "advantage" in giving fructose by mouth than by the intravenous route.

RÔLE OF THE LIVER IN FRUCTOSE METABOLISM

The liver plays an important rôle in carbohydrate metabolism and this is particularly true for fructose. To obtain more direct information in man hepatic vein catheterizations were performed in diabetic and nondiabetic subjects,[4] splanchnic blood flow being determined by the bromsulfalein technique. Samples of femoral artery and hepatic vein blood were drawn at frequent intervals, and estimates of splanchnic balances of glucose, fructose, and pyruvic acid were calculated by multiplying the appropriate arterial-

TABLE 6. ARTERIAL AND PORTAL ANASTOMOTIC VEIN CONCENTRATIONS OF FRUCTOSE AND GLUCOSE FOLLOWING ORAL ADMINISTRATION 50 GRAMS OF FRUCTOSE

	Portal anastomotic vein mg./100 ml.	*Femoral artery mg./100 ml.*	*Difference in concentration mg./100 ml.*	*"Conversion" per cent*
30 minutes after ingestion				
Fructose	43	20	23	
Glucose	121	116	5	18
60 minutes after ingestion				
Fructose	47	20	27	
Glucose	130	125	5	16

hepatic venous blood concentration difference by the corresponding estimated splanchnic blood flow. After control samples were collected, 10 per cent glucose or fructose was infused into a peripheral vein at such a rate that one gram of hexose per kilogram of body weight was given in one hour. As shown in Table 7, fructose was taken up by the splanchnic system twice as rapidly as was glucose in the nondiabetic subject, and this ratio was maintained in the diabetic state in the absence of exogenous insulin effect. Even in severe diabetic ketosis the ability of the liver (splanchnic system) to take up fructose remains unimpaired. This more rapid uptake of fructose by the liver is an important factor in the more rapid disappearance of fructose from the blood stream described in the previous sections.

Studies of pyruvic acid splanchnic balance in these cases showed that the normally slow uptake of pyruvic acid was reversed when either glucose or fructose was infused. However, the output was equivalent to only 0.5 per

cent of the hexose uptake when glucose was given and to 3.6 per cent when fructose was administered. Consequently, at least part of the greater peripheral concentration of pyruvic acid that occurs when fructose is given is the result of this output by the liver.

UPTAKE OF FRUCTOSE BY MUSCLE

The utilization of fructose by muscle tissue has been studied almost entirely in species other than man. Van Itallie[81] in 1953 measured fructose uptake in the forearm (composed 80 per cent of muscle) of 10 nondiabetic

TABLE 7. PERCENTAGE OF ADMINISTERED HEXOSE (1.0 GM./KG.) ASSIMILATED BY SPLANCHNIC SYSTEM DURING HOUR OF INFUSION

Subject	*No. of cases*	*Per cent uptake of hexose infused*	
		Glucose	*Fructose*
Nondiabetic	6	19	..
	4	..	37
Diabetic (minimal or no ketosis)	5	19	..
	4	..	42
Diabetic (severe ketosis)	1	0	..
	2	..	48

subjects. After injection of a priming dose of between 25 and 30 gms. of fructose administered intravenously as a 10 per cent solution in approximately a 30-minute period, the infusion was continued at a constant rate of 0.58 gm./min. Capillary and venous bloods were taken at 15-minute intervals for one hour beginning 30 to 100 minutes from the start of the experiment and the arterio-venous difference of fructose was determined. At an average arterial level between 66.1 and 68.9 mg./100 ml. the A-V difference ranged between 10.6 and 13.4 mg./100 ml., even up to two and a half hours from the beginning of the infusion. Using the same approach, modified so that 30 gms. of fructose was given in the first 30 minutes and then continued at 0.58 gm./min. for two and a half hours, we found a similar uptake of fructose in three experiments on stable diabetic subjects. Figure 6 shows the results in one of these tests. It can be seen that at the end of 150 minutes the A-V difference was more than 7 mg./100 ml. In an experiment on another diabetic patient, the arterial level for fructose under these conditions at the end of 75 minutes was 30 mg./100 ml. higher than that in the vein. Another priming dose was then given, and the rate of infusion was

doubled to 1.2 gm./min. At the higher arterial levels obtained (225-250 mg./100 ml.) the A-V difference immediately increased and at the end of two and a half hours was 70 mg./100 ml. The magnitude of the amount taken up after such a long period of infusion suggests the conclusion that direct assimilation ("utilization"?) by muscle cells must have been taking place.

OXIDATION OF FRUCTOSE IN VIVO AS MEASURED WITH ISOTOPES

The whole problem of fructose metabolism in man is complicated by the fact that knowledge of its utilization by various tissues is still not sufficiently detailed. Ultimately these data will become available and the sum total of its metabolism can then be determined. Lacking this information, we have turned to the study of the oxidation *in vivo* of C^{14} fructose. Ninety μc of evenly labeled C^{14} fructose without carrier were injected intravenously into normal and diabetic subjects, and the specific activity of CO_2 in the expired air was measured at frequent intervals for a period of at least eight hours. In Figures 7 and 8 these results are compared with the specific activity of CO_2 in the expired air following the intravenous injection of the same amount (90 μc) of uniformly labeled glucose-C^{14} in the *same* normal or diabetic subject on another occasion. After fructose in the diabetic subject (Fig. 8) a peak activity was attained very rapidly in not more than 10 or 15 minutes, and in this instance this was even faster than that achieved in the normal subject (Fig. 7). In contrast, the peak activity of CO_2 after glucose in the diabetic patient was significantly delayed as compared with the nondiabetic subject. It appears, therefore, that there is a rapid metabolic transport of fructose to the CO_2 pool and that there is no significant reduction in this rate in diabetes even in the absence of insulin. The oxidation of fructose to carbon dioxide does not seem to be under the control of insulin.

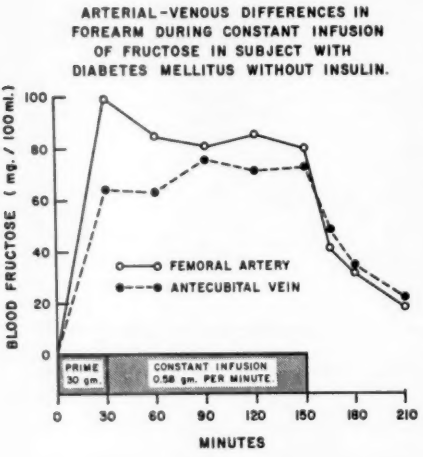

FIG. 6.

COMPARISON OF FRUCTOSE AND GLUCOSE IN VARIOUS CLINICAL STATES ASSOCIATED WITH DISTURBANCES IN CARBOHYDRATE METABOLISM

Trauma and infection

Because of the well-documented effects of stress situations on glucose metabolism, a series of experiments was performed in nondiabetic human subjects in order to compare the manner in which fructose was handled in the same circumstances.[9] Five patients scheduled for elective herniorrhaphies under spinal anesthesia and six patients scheduled for "major" surgery (gastrectomy, etc.) under general anesthesia were given intra-

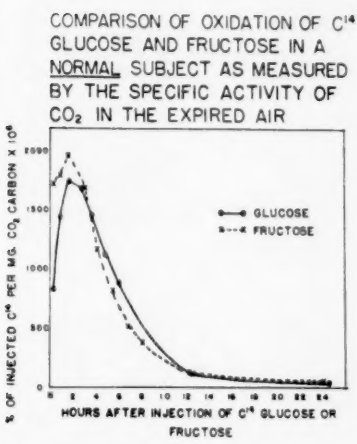
COMPARISON OF OXIDATION OF C14 GLUCOSE AND FRUCTOSE IN A NORMAL SUBJECT AS MEASURED BY THE SPECIFIC ACTIVITY OF CO2 IN THE EXPIRED AIR
GLUCOSE
FRUCTOSE
% OF INJECTED C14 PER MG. CO2 CARBON X 10⁶
HOURS AFTER INJECTION OF C14 GLUCOSE OR FRUCTOSE

FIG. 7.

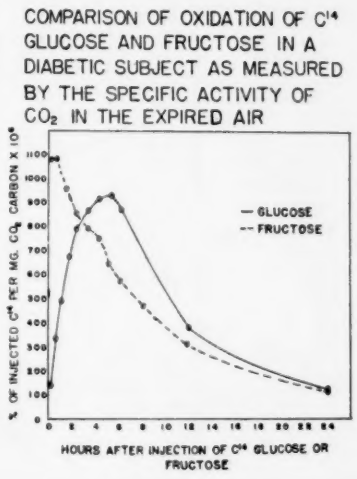
COMPARISON OF OXIDATION OF C14 GLUCOSE AND FRUCTOSE IN A DIABETIC SUBJECT AS MEASURED BY THE SPECIFIC ACTIVITY OF CO2 IN THE EXPIRED AIR
GLUCOSE
FRUCTOSE
% OF INJECTED C14 PER MG. CO2 CARBON X 10⁶
HOURS AFTER INJECTION OF C14 GLUCOSE OR FRUCTOSE

FIG. 8.

venous glucose and fructose (1.0 gm. per kg. in one hour as a 10 per cent solution) on successive days pre-operatively, and these were repeated on the first and second post-operative days. All four tolerance tests were done in each patient. The order in which the glucose and fructose was given was alternated from case to case. The results of these tolerance tests are shown in Table 8. Even the relatively simple trauma of the herniorrhaphy operation was sufficient to elevate the peak value of glucose at the end of the infusion and result in a delayed fall. In the major surgical cases the lag in the fall of glucose was even more evident (58 mg./100 ml. above control values at 180 minutes compared to 34 mg./100 ml. in the herniorrhaphy operations). The fructose tolerance tests in contrast were essentially unaltered by the procedure.

Similarly, a patient with typhoid fever studied during the height of the febrile phase showed an alteration in glucose tolerance as compared with the test after recovery, while again the fructose tolerance curves were both normal and identical (Fig. 9).

The mechanisms by which stress situations alter the metabolism of glucose are still being vigorously debated. It is tempting to speculate that,

GLUCOSE AND FRUCTOSE TOLERANCE IN TYPHOID FEVER

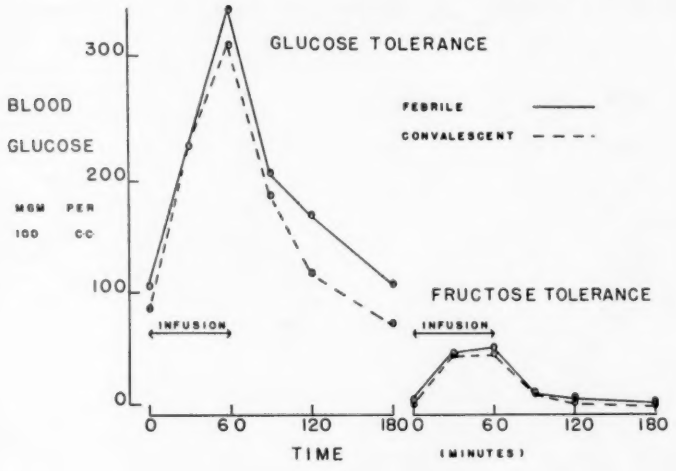

FIG. 9.

if stress results in a stimulation of the pituitary-adrenal axis (Selye) and the hormones released inhibit the action of glucokinase[25] resulting in a delay in the entrance of glucose into the metabolic pool, the normal disappearance curves of fructose could then be explained by the fact that fructose has its own separate fructokinase not influenced by these hormones. On the other hand, if insulin acts upon a cellular membrane system to accelerate transport of glucose[13, 21, 22] but has no effect on the entrance of fructose into the cell, it must be assumed that the stress must have some inhibitory effect on the factor or factors responsible for glucose transport but does not affect the permeability of the cell wall to fructose.

Anesthesia

General anesthetics, particularly ether, have been reported to elevate the blood sugar and diminish the utilization of carbohydrate.[24] Twelve volunteer

TABLE 8. THE EFFECTS OF SURGERY ON INTRAVENOUS GLUCOSE AND FRUCTOSE TOLERANCE (1.0 GM./KG./1 HR.) BEFORE AND AFTER OPERATION

	Herniorrhaphies Average of 5 subjects				*Major surgery Average of 6 subjects*			
	Glucose tolerance		*Fructose tolerance*		*Glucose tolerance*		*Fructose tolerance*	
Time minutes	*Pre-op. blood glucose mg./100 ml.*	*Post-op. blood glucose mg./100 ml.*	*Pre-op. blood fructose mg./100 ml.*	*Post-op. blood fructose mg./100 ml.*	*Pre-op. blood glucose mg./100 ml.*	*Post-op. blood glucose mg./100 ml.*	*Pre-op. blood fructose mg./100.ml.*	*Post-op. blood fructose mg./100.ml.*
0	91	93	1	2	93	132	2	3
60	311	360	61	54	322	375	83	81
120	107	157	10	9	142	190	14	12
180	69	103	4	5	78	136	4	6

subjects hospitalized for rehabilitation at Highland View Hospital were given ether anesthesia without surgery for a period of 90 minutes.[7] Anesthesia was induced by the intravenous administration of 150-300 mg. of thiopental sodium in order to minimize the excitement and physical activity frequently encountered during the induction of ether narcosis. Thirty minutes after surgical anesthesia was achieved, a specimen of blood was drawn and 1 gm./kg. of a 10 per cent solution of glucose (six subjects) or

TABLE 9. EFFECTS OF ETHER ANESTHESIA ON FASTING BLOOD GLUCOSE AND FRUCTOSE LEVELS AND ON INTRAVENOUS GLUCOSE AND FRUCTOSE TOLERANCES

No. of subjects	*Glucose tolerance*			*Fructose tolerance*		
	6	*6*	*3*	*6*	*6*	*4*
Time minutes	*Control blood glucose mg./100 ml.*	*During anesthesia blood glucose mg./100 ml.*	*24 hours after anesthesia blood glucose mg./100 ml.*	*Control blood fructose mg./100 ml.*	*During anesthesia blood fructose mg./100 ml.*	*24 hours after anesthesia blood fructose mg./100 ml.*
—30		91*			1.5*	
0	93	133	92	1.3	1.5	1.6
60	309	396	315	90.5	99.9	96.7
120	144	199	158	12.2	19.0	13.3
180	82	137	74	5.2	12.7	7.3

* Pre-anesthesia values obtained in four of six subjects tested.

fructose (six subjects) was infused intravenously at a constant rate for one hour, at the end of which time both the infusion and the ether were discontinued. Similar tolerance tests were done as controls in each subject one or more days preceding the anesthesia and 24 hours afterwards (three and four subjects in each group, respectively). The effect on blood glucose and fructose levels of the first 30 minutes of anesthesia alone was studied in four subjects in each group. The results of these tests are shown in Table 9. Ether anesthesia (30 minutes) increased the fasting blood glucose significantly from 91 to 133 mg./100 ml. but did not alter the fasting blood fructose level. The glucose tolerance test also showed definite impairment during anesthesia, but this effect was gone by the next day. There was a slight delay in the return of the blood fructose as compared with the control curve, but this was only a fraction of that seen with glucose (7.5 mg./100 ml. compared with 55 mg./100 ml. above control values at 180 minutes, respectively).

Starvation diabetes

The phenomenon of starvation diabetes, or the induction of impaired carbohydrate tolerance by the restriction of dietary carbohydrate, was first described in 1874 when Lehmann[14] found that the injection of sugar into the mesenteric veins produced greater glycosuria in fasted than in fed dogs. The same alteration in glucose tolerance after deprivation or reduction in carbohydrate intake has been repeatedly shown in man. Peters,[22] in his comprehensive review of this problem in 1945, concluded that the available evi-

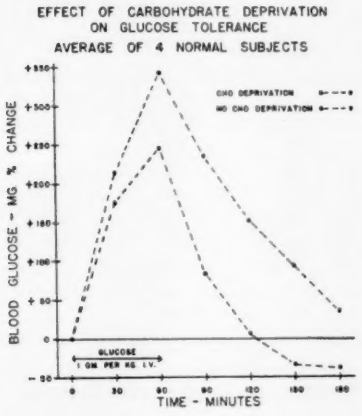

FIG. 10.

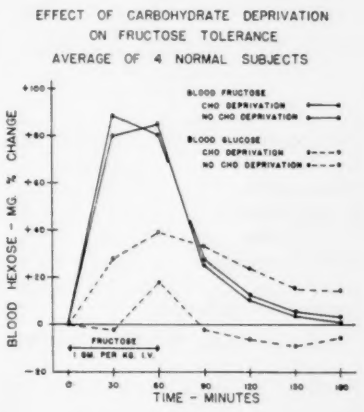

FIG. 11.

dence at the time pointed to "defective combustion" as the cause of this impairment. The study of fructose metabolism in states of carbohydrate deprivation was undertaken in man to determine where the possible sites of the block in "combustion" might exist. Intravenous glucose tolerance tests were performed on four normal human subjects who had previously been on adequate diets. After two days of fasting (two cases) or two days during which the diet consisted of meat and butter only (two cases), the tolerance tests were repeated (Fig. 10). Under similar circumstances at another time, fructose tolerance tests were performed on the same four subjects (Fig. 11). The rate of removal of administered fructose was not found to be significantly altered. However, the blood glucose rise associated with fructose administration was greater after carbohydrate restriction than in the well-fed state, and the return of the blood glucose to the initial level was delayed after dietary restriction. These results afford further evidence that the metabolism of fructose differs from the metabolism of glucose in man

and indicate that the block in glucose utilization following carbohydrate deprivation involves one (or more) of the reactions between glucose and the entry of fructose into the glycolytic scheme. The findings are consistent with the hypothesis of Wyshak and Chaikoff[28] that the glucokinase reaction is impaired by fasting, while the phosphorylation of fructose, which is under the influence of a separate enzyme (fructokinase), is not altered during starvation.

The importance of these observations in the management of clinical diabetes seems clear. The alterations in carbohydrate tolerance in diabetes produced by trauma, infection, or starvation are much more marked than

TABLE 10. BLOOD ANALYSES AT BEGINNING OF TREATMENT

		Fructose	*Glucose*	*No CHO*
Blood sugar	mg./100 ml.	700	488	794
N.P.N.	mg./100 ml.	56	51	40
Serum acetone	mg./100 ml.	64	62	42
Serum chloride	mEq./l.	82	91	85
Serum sodium	mEq./l.	121	128	120
Serum potassium	mEq./l.	7.0	5.9	6.0
CO_2	vol.%	33	39	42
Hours since last insulin		42	41.5	47.5

in normal subjects. Since, under these circumstances, parenteral fluids are often necessary, the evidence accumulated so far indicates the value of fructose as the carbohydrate of choice in management.

THE METABOLISM OF FRUCTOSE IN DIABETIC ACIDOSIS AND ITS USE IN EARLY TREATMENT

The physiological changes usually accompanying diabetic acidosis provide theoretical reasons for using fructose in therapy. There is considerable evidence to indicate that the patient in acidosis is under "stress" either "spontaneously"[18] or as a result of trauma or infection that may have precipitated the acidosis. Undoubtedly, there exists a state of insulin "resistance" and insulin, accordingly, is less effective early in treatment. Field and Stetten[9] have demonstrated recently the presence of an insulin antagonist in diabetic acidosis, disappearing within hours after treatment is instituted. This antagonist was nondialyzable and migrated electrophoretically with the alpha-globulin fraction of the serum proteins. Peters[28] has emphasized that diabetic acidosis is almost invariably associated with carbohydrate starvation and has summarized the evidence for using glucose even in the initial

stage. Later, however, Seldin and Tarail[27] reported on 17 cases of diabetic acidosis receiving moderate or massive injections of glucose early in treatment. They found that hyperglycemia resulted in an increase of the effective osmotic pressure of the extracellular fluid, producing cellular dehydration. The administration of glucose, even though it might replenish glycogen stores and facilitate the combustion of carbohydrate, enhanced the cellular dehydration and perpetuated the polyuria because of the increased excretion of glucose in the urine. Other authors[9, 26] have strongly opposed the use of carbohydrate. Since fructose does not require insulin for its metabolism, is more rapidly removed from the blood stream, is a better glycogen former than glucose,[15] and its metabolism is not affected by stress states or starva-

TABLE 11. TREATMENT IN FIRST SIX HOURS

Experiment	*Insulin units*	*Fluids ml.*	*NaCl mEq.*	*CHO gm.*
I	350	3300	482	175 Fructose
II	350	3250	475	175 Glucose
III	350	3250	475	No CHO

tion, it was logical to assess its value in the early treatment of diabetic acidosis under controlled conditions.[18]

Experimental acidosis was produced by the withdrawal of insulin in a human volunteer on three separate occasions. Within 40 to 50 hours after the last dose of regular insulin, clinical acidosis regularly ensued, with serum ketone levels of over 40 mg./100 ml. and blood sugars over 450 mg./100 ml. Complete balance studies were done during the withdrawal, treatment, and recovery periods. During insulin withdrawal periods all exogenous carbohydrate derived from protein and fat catabolism were excreted almost quantitatively. The results of the blood analyses at the beginning of treatment in each of the three experiments is shown in Table 10.

The period of study described here covered the first six hours of treatment. The amounts of insulin, water, and NaCl were similar in each experiment, with hexose as the variable to be investigated (Table 11).

One hundred units of insulin were given initially and 50 units each hour thereafter for a total of 350 units in the six-hour period. Approximately 475 mEq. of NaCl was given as normal saline in each of the three experiments. In Experiment I, 75 grams of fructose were given intravenously as a 10 per cent solution in the first hour, and thereafter 20 gm. each hour for a six-hour total of 175 gm. In the second experiment glucose was given in

similar amounts and at the same rate. In the third experiment saline alone was used as the constituent of the fluids. Solutions were combined in such a way that the total water administered was given at the same rate and amount in each test. Figure 12 shows the changes in blood glucose concentrations during therapy. For purposes of comparison the levels are referred to the initial blood glucose concentrations as zero. When no exogenous carbohydrate was given, the blood glucose fell at an approximately constant rate. After 75 gm. of glucose there was an initial rise at one hour of approximately 125 mg./100 ml. and then a steady fall when the amount of

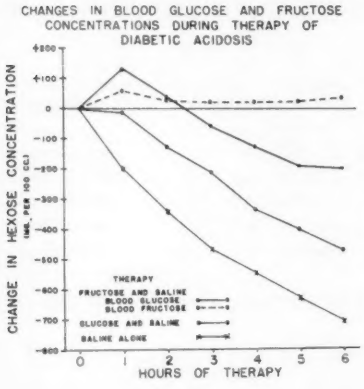

FIG. 12.

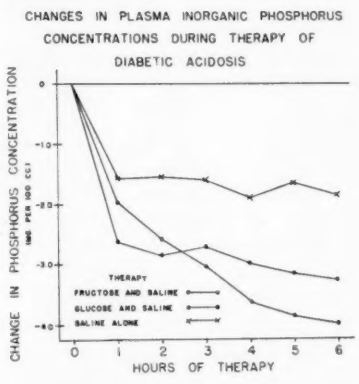

FIG. 13.

glucose infused was reduced to 20 gm. per hour. After 75 gm. of fructose there was a slight fall in blood glucose at the end of the first hour and then a rapid fall at approximately the same rate as after saline alone. Blood fructose levels were found to be in the same range as to be expected in normal subjects, the highest level being less than 75 mg./100 ml.

Carbohydrate balances for the first six hours were as follows: in Experiment I the administration of 175 gm. of fructose resulted in a net balance of 163 gm.; with glucose administration the net balance was 124 gm.; while with saline alone the total carbohydrate either stored or utilized measured only 18 gm. The excretion of fructose as such in the urine in Experiment I amounted to less than 7 gm.

Plasma inorganic phosphorus fell least after saline and most after fructose (Fig. 13). The administration of carbohydrate did not result in any increased excretion of either sodium or potassium in the urine during treat-

ment (Table 12). Since potassium was not included in the treatment schedule in the first six hours, serum potassium levels fell (Fig. 14), the fall being approximately the same in each instance. After fructose administration there was evidence of some transference of potassium into the cells which was lacking in the other two experiments.

TABLE 12. EFFECT OF HEXOSE THERAPY IN DIABETIC ACIDOSIS ON SODIUM AND POTASSIUM EXCRETION IN THE URINE

(First six hours)

	Therapy		
	Fructose	*Glucose*	*No CHO*
Sodium mEq.			
Intake I.V.	482	475	475
Output in urine	40	36	43
Potassium mEq.			
Intake I.V.	0	0	0
Output in urine	21	18	20

TABLE 13. CHANGES IN CO_2 COMBINING POWER DURING FIRST SIX HOURS OF THERAPY WITH AND WITHOUT ADDED HEXOSE

Experiment no.	*Hexose used*	*Co_2 value (Vol./100 ml.)*		
		initial	*final*	*increase*
I	Fructose	33	52	19
II	Glucose	39	56	17
III	No CHO	42	59	17
IV	Fructose	29	51	22

Urinary volume was definitely greater with glucose therapy, but fructose, because of the much smaller excretion of hexose in the urine, caused only a slightly greater water output as compared with the control regime. Serum ketone levels fell on the average about 25 per cent faster when carbohydrate was administered. No significant difference was found between fructose and glucose.

Some writers have suggested that recovery from the acidosis would be delayed with fructose therapy because of the markedly increased rate of formation of pyruvic and lactic acid in the blood. Data pertinent to the discussion were obtained in these three experiments and on a fourth occasion

when fructose again was used in treatment under similar controlled conditions (Table 13). The results show that the use of fructose actually increases the rate of recovery from acidosis, a conclusion that might have been anticipated from the known facts of fructose metabolism.

This same plan of experiment has also been applied to cases of clinical diabetic acidosis admitted to the University Hospitals during a period of 13 years. Patients entering in acidosis without known complicating factors were treated according to the schedule in Table 11. Seven cases were treated without carbohydrate, 5 with fructose, and 16 with glucose. The effects in mg./100 ml. per hour, on excretion of carbohydrate, on carbohydrate utilized or stored, on blood ketone fall, and on water balance are summarized in Table 14.

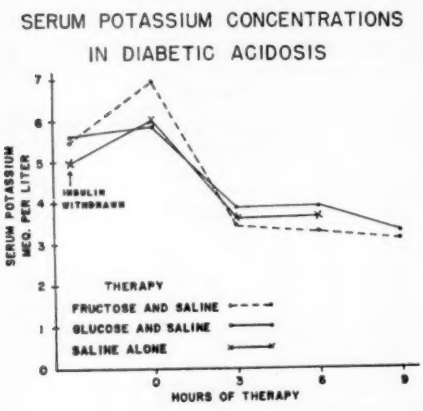

FIG. 14.

It is apparent that the results in the treatment of clinical diabetic acidosis are similar to those found in the experimentally induced type. With fructose therapy, *other factors being equal,* there are significant advantages, as measured by carbohydrate and water balances, and a more rapid fall in blood ketones. There is no delay in the recovery from acidosis, despite the increased production of pyruvic and lactic acids.

SUMMARY

The metabolism of fructose in normal and diabetic human subjects has been reviewed. The blood fructose tolerance test has been shown to be unaltered in diabetic subjects even in the absence of exogenous insulin. Fructose produces a much greater rise in blood pyruvic acid as compared with glucose, and this rise is the same or greater in diabetes. In hepatic vein catheterization studies, the livers of both normal and diabetic subjects remove intravenously administered fructose twice as rapidly as they do glucose in comparable situations. A considerable portion of administered fructose is converted in the body, but the "net advantage" of fructose over glucose in diabetic subjects is between 20 per cent and 25 per cent. Oral

administration is less advantageous, probably because of further conversion of fructose to glucose by the intestinal mucosa during absorption. It has been shown that stress situations (trauma, infection), ether anesthesia, and starvation states do not depress fructose metabolism as they do glucose. Studies in diabetic acidosis indicate the value of fructose in the initial hours of therapy.

TABLE 14. EFFECT OF HEXOSE IN TREATMENT OF DIABETIC ACIDOSIS
(First six hours)

	No CHO	*Glucose*	*Fructose*
Number of cases	7	16	5
Blood sugar fall mg./100 ml./hr.	84	11	66
Excretion CHO gm./6 hrs.	30	116	60
CHO "utilized" gm.	18	63	147
Blood ketone fall mg./100 ml./hr.	5.3	6.6	6.9
Water balance ml./6 hrs.	1970	650	1900

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SIGNIFICANCE OF BLOOD SUGAR AND SERUM ELECTROLYTE CHANGES IN CIRRHOSIS FOLLOWING GLUCOSE, INSULIN, GLUCAGON, OR EPINEPHRINE§

It has been established that the administration of glucose, insulin, or epinephrine to control and experimental subjects produces a decrease in serum inorganic phosphorus and, less consistently, in serum potassium[1-4, 9, 12-16, 18-21, 23-29, 34, 35, 37-43, 47, 49-54, 56, 57, 59, 60, 64, 66] in addition to the change in blood sugar levels. Cirrhosis is known to affect the blood sugar responses to alterations of carbohydrate metabolism by these agents,[5, 10, 22, 30, 44, 46, 67] but data on associated electrolyte changes are few, limited to measurements of serum inorganic phosphorus and of potassium, and often inconclusive. The studies herein described indicate that changes in other electrolytes may also occur following glucose, insulin, glucagon, or epinephrine administration and that their direction and magnitude may serve as an index of alterations in carbohydrate metabolism induced by these agents.

MATERIALS AND METHODS

Glucose (0.5 gram/kilogram) and crystalline insulin (0.1 unit/kilogram), alone or in combination, and glucagon (16 gamma/kilogram) were rapidly administered by vein to healthy young adults or patients hospitalized with cirrhosis. Epinephrine (0.01 milliliter/kilogram of a 1:1000 solution) was injected in divided dosage subcutaneously at 0, 10, and 20 minutes to similar groups of subjects. Venous blood withdrawn with minimum stasis prior to and at intervals following the above injections was analyzed for concentrations of sugar in blood and for levels of inorganic phosphorus, potassium, total carbon dioxide, chloride, and sodium in serum.[11]

The presence of cirrhosis was established in each instance by needle biopsy of the liver in addition to the demonstration of the characteristic abnormalities of serum proteins, decreased bromsulphalein excretion, elevated bilirubin levels, etc. The tolerance studies were conducted once convalescence from hepatic decompensation had been

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TABLE 1. AVERAGE DISAPPEARANCE RATE OF EXTRACELLULAR GLUCOSE (MGM./MINUTE/KG.) DURING GLUCOSE TOLERANCE AND INSULIN-GLUCOSE TOLERANCE TESTS IN ADULT CIRRHOTICS COMPARED TO HEALTHY CONTROLS

	Controls		*Cirrhotics*	
	0-30 min. Mean ± S.D. (♯) *[Range]*	*0-60 min. Mean ± S.D.* (♯) *[Range]*	*0-30 min. Mean ± S.D.* (♯) *[Range]*	*0-60 min. Mean ± S.D.* (♯) *[Range]*
Glucose tolerance test 0.5 g./kg. i.v. at zero time	12.23 ± 2.2 (11) [8.1 — 15.2]	8.02 ± 0.8 (11) [6.9 — 8.9]	*9.59 ± 2.1 (19) [6.4 — 14.1]	*6.35 ± 1.2 (19) [3.9 — 8.2]
Insulin-glucose tolerance test 0.1 u. insulin/kg. i.v. 0.5 g. glucose/kg. i.v. at zero time	15.30 ± 1.5 (6) [13.1 — 17.6]	9.4 ± 0.8 (6) [8.0 — 10.0]	14.41 ± 2.9 (9) [8.9 — 17.7]	8.84 ± 0.7 (9) [7.5 — 9.9]

* Significantly less than in the healthy adult controls (p values of 0.003 and 0.00025 in the 0-30- and 0-60-minute groups, respectively).

well established, i.e., alcoholic intake had been stopped, food intake resumed, and ascites and edema largely or entirely delivered.

Rates of glucose disappearance in cirrhotics and in control subjects have been estimated for the intervals between 0 and 30 and 0 and 60 minutes following glucose and glucose-insulin administration, assuming that glucose was distributed in a volume of extracellular fluid equivalent to 20 per cent of the body weight. Calculations were made in accordance with the usual formula save that the correction for the higher glucose concentration in extracellular fluid has been omitted:

$$\text{Bl.S. (mgm. per liter)} \times \text{ECW (liters)} + \text{Injected glucose in milligrams} = \text{Total Extracellular Glucose or mgm. Glucose}_{ECW}$$

Then:

$$\frac{\text{mgm. Glucose}_{ECW_{Start}} \text{ minus mgm. Glucose}_{ECW_{Finish}}}{\text{Time}_{(\text{minutes})}} \div \text{B.Wgt. (kgm.)} = \text{Average Disappearance Rate (mgm./min./kgm.)}_{\text{during given interval}}$$

Similar calculations for glucagon, insulin, and epinephrine were not attempted because the magnitude of the increment of glucose from hepatic glycogen in the case of the first and from hepatic and/or tissue glycogen in the last two cannot be measured. In the glucose and glucose-insulin calculations it was assumed that the increment in glucose load came from exogenous sources only.

A. *Changes in blood sugar and serum electrolytes following intravenous glucose*

The rapid intravenous injection of glucose, 0.5 gram/kilogram of body weight, produced a rise in the blood sugar levels of adult patients with cirrhosis which was greater and more prolonged than that seen in healthy young adults under similar circumstances. Calculation of the average rate of glucose disappearance showed that in keeping with the hyperglycemia the cirrhotic patients disposed of less glucose during the 0 to 30- and 0 to 60-minute intervals (Table 1). This relatively greater hyperglycemia was accompanied by a more pronounced and more protracted decrease in serum inorganic phosphorus (Fig. 1). With the exception of the serum bicarbonate levels, the other electrolytes (potassium, sodium, and chloride) showed no comparable change. In the normals the bicarbonate tended to rise while in the cirrhotics it fell.

B. *Responses to intravenous insulin*

The intravenous administration of insulin to control adult subjects produced a distinct decrease in blood sugar, serum inorganic phosphorus, bicarbonate, and potassium, and a rise in chloride and sodium. These findings are shown in Figure 2. Similar amounts of insulin given to adult cirrhotics produced less of a decrease in blood sugar and in serum inorganic phosphorus. The statistically significant differences are indicated in the figure. The changes in the levels of potassium, bicarbonate, sodium, and chloride were comparable in direction and in degree in the two groups.

C. *Effects of glucose and insulin in combination*

The blood sugar levels followed a similar pattern in the cirrhotic and in the control subjects. Except as marked, the visible differences are not statistically significant. The average rates of glucose disappearance between 0 to 30 and 0 to 60 minutes are vir-

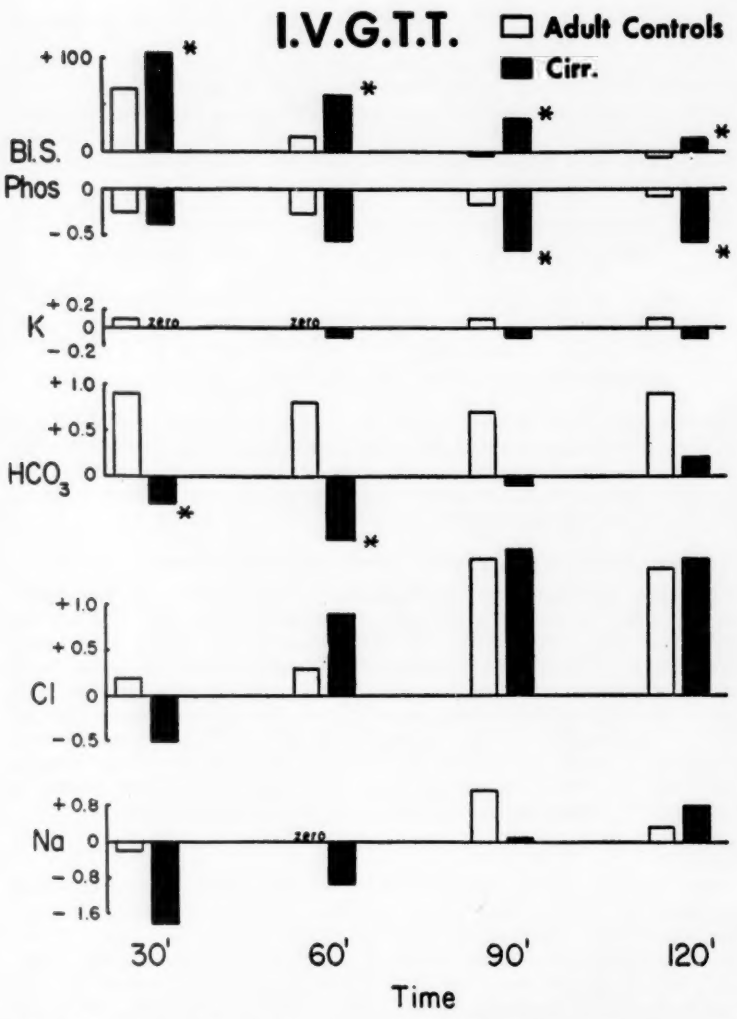

FIG. 1. Intravenous glucose tolerance test in cirrhosis. Glucose (0.5 gram per kilogram of body weight in three to five minutes at zero time) produced greater hyperglycemia and ultimately a greater decrease in phosphorus in these 19 patients with cirrhosis. Statistically significant differences are indicated by asterisks. The decrease in serum total CO_2 content (expressed as HCO_3) in the cirrhotics reflects an unidentified disturbance in anion-cation balance. Blood sugar and serum phosphorus changes are expressed in mgm.%; all others in mEq. per liter.

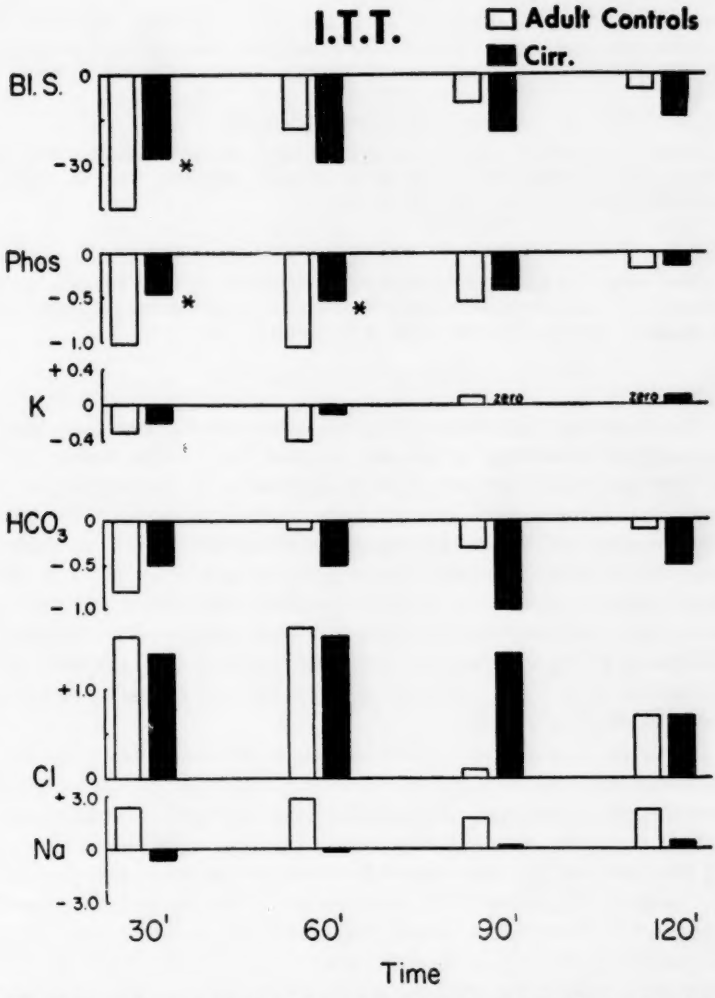

FIG. 2. Lesser hyperglycemia and lesser hypophosphatemia following insulin administration in cirrhosis. Crystalline insulin (0.1 unit per kilogram) was administered intravenously at zero time to 11 cirrhotics and 9 healthy adults. The response of the cirrhotics is indicated by solid columns.

tually identical (Table 1) and hence in keeping with the similarity of the blood sugar curves. The phosphorus drop at 60 minutes was of lesser degree in the cirrhotics than in the controls. The bicarbonate fell early in the cirrhotic group in contrast to the uniformly persistent increases in the healthy adults. These changes and the statistically significant differences in the sodium and chloride responses are shown in Figure 3.

D. *Blood sugar and electrolyte levels following glucagon*

In four adult cirrhotics only the rise in blood sugar and the associated decrease in serum inorganic phosphorus appear to be of lesser magnitude than the changes observed in healthy adults (Figs. 4a and 4b).

E. *Epinephrine hyperglycemia and associated changes*

From Figure 5 it is evident that in cirrhosis epinephrine produced less of a rise in venous blood sugar, less of a decrease in serum potassium, phosphorus and bicarbonate levels, and a decrease rather than a rise in the serum chloride.

DISCUSSION

The discussion which follows is predicated on certain assumptions based on available information.[10] It has been assumed, for example, that a carbohydrate load which does not result in glycosuria can be disposed of by deposition as liver or tissue glycogen or by anaerobic glycolysis within cells. Also, insulin without an accompanying carbohydrate load accelerates anaerobic glycolysis, increases muscle glycogen, and, when given in sufficient amounts, depletes liver glycogen. Insulin in combination with glucose on the other hand augments anaerobic glycolysis and replenishes both liver and tissue glycogen. Finally, it has been assumed that glucagon and epinephrine each result in hepatic glycogenolysis while only epinephrine decreases muscle glycogen.

Insofar as accompanying electrolyte changes are concerned it has been postulated that anaerobic glycolysis is accompanied by a decrease in serum inorganic phosphorus and, demonstrable under appropriate conditions, by a fall in serum potassium. In keeping with findings on analysis of tissue it has been assumed that deposition of liver glycogen preëmpts potassium but not inorganic phosphorus.[10] The concomitants of muscle and other tissue glycogen formation are not known. Only speculation is possible concerning the mechanisms whereby levels of the other electrolytes are altered. Though water shifts undoubtedly occur, as in glucose loading or removal, it cannot be the sole factor because the changes in the individual electrolytes are not always in the same direction. It is reasonable to suppose that variations in serum total CO_2 content reflect net anion-cation shifts, i.e., accumulation or removal, though primary respiratory adjustments and, to a lesser extent,

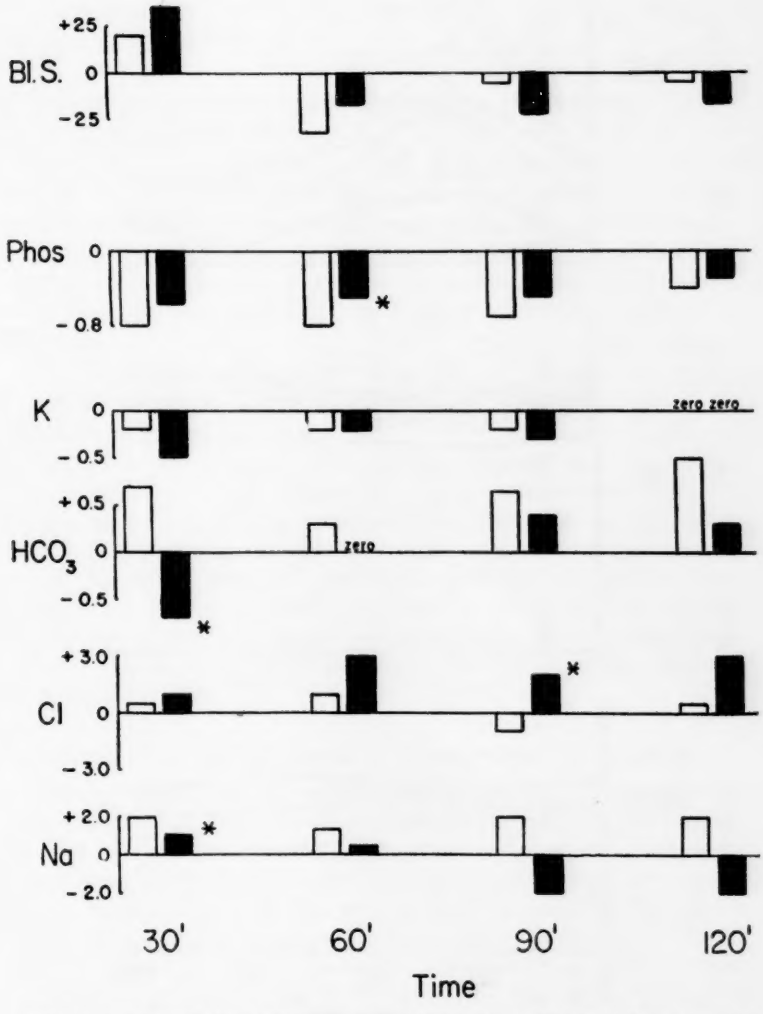

FIG. 3. Insulin and glucose tolerance tests. Glucose (as in Fig. 1) followed immediately by crystalline insulin (as in Fig. 2) produced identical responses in 9 cirrhotics (solid columns) and in 6 controls.

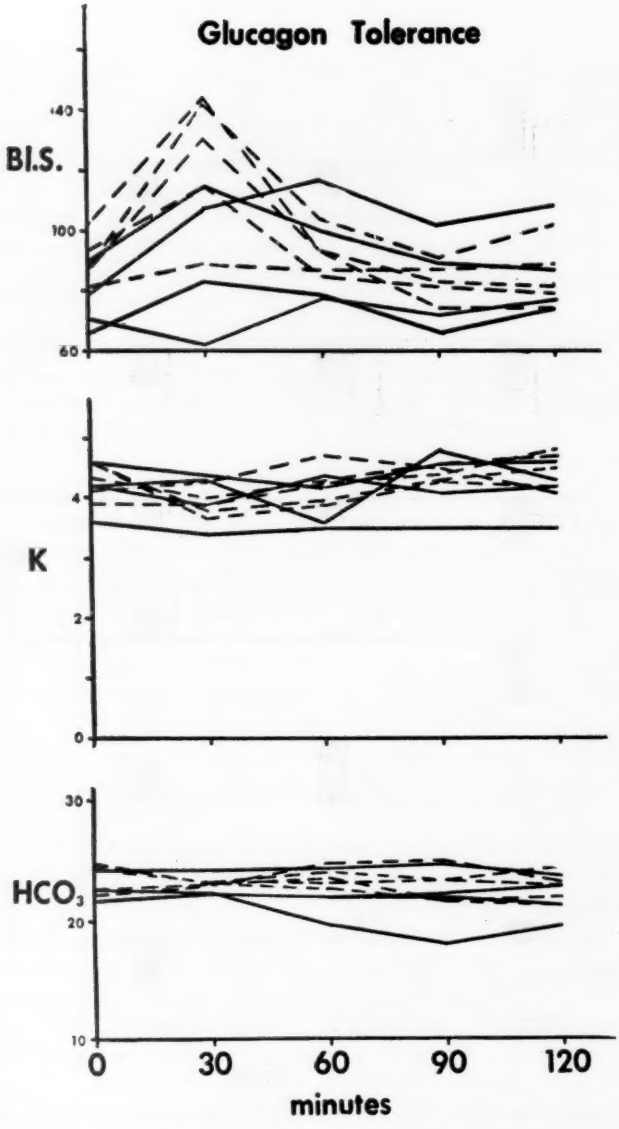

FIG. 4a.

FIGS. 4a and 4b. Changes in blood sugar and serum electrolyte following glucagon. Glucagon, 16 gamma per kilogram of body weight, was given at zero time with lesser hyperglycemia and a smaller decrease in the serum inorganic phosphorus in the patients with cirrhosis (solid lines).

renal adjustments may play a rôle. The mechanisms of the chloride and sodium changes, when they do occur, remain obscure but may well represent ion exchange phenomena.

A. CHANGES COMPATIBLE WITH DECREASED ABILITY OF THE CIRRHOTIC TO LAY DOWN LIVER GLYCOGEN

There were clear-cut differences between the blood sugar responses of the cirrhotic and the healthy adult to intravenous glucose, intravenous insulin, intravenous glucagon, and subcutaneous epinephrine. The greater hyperglycemia following glucose, the lesser hypoglycemia after insulin, and the lesser blood sugar rises in response to glucagon and epinephrine are all in keeping with the recognized diminished ability of the cirrhotic to store liver glycogen.

B. EVIDENCE OF GREATER ANAEROBIC GLYCOLYSIS IN CIRRHOTICS PRESENTED WITH A GLUCOSE LOAD

The serum inorganic phosphorus changes during these intravenous glucose tolerance tests in cirrhotics show enhanced peripheral disposal of glucose by processes involving phosphorylation. This could be explained by the relatively greater carbohydrate load presented to extrahepatic tissues by virtue of decreased deposition of glucose as liver glycogen. Such an interpretation is in keeping with the demonstration by Pollack and his colleagues that the major portion and perhaps all of the loss of serum inorganic phosphorus following glucose administration is related to anaerobic glycolysis in extrahepatic tissues.[48, 51, 68]

C. EXISTENCE OF A POSSIBLE ANTI-INSULIN FACTOR OR EFFECT IN CIRRHOTICS

However, defective deposition or release of glycogen cannot explain the diminished hypoglycemic-hypophosphatemic-hypokalemic action of insulin in cirrhosis. It might therefore be postulated that at least the immediate effectiveness of exogenous insulin is partially cancelled in patients with cirrhosis. Such inhibition of insulin action could be mediated through the insulinase-anti-insulinase system studied by Mirsky and by others[45, 65] or it might be related to the insulin resistance encountered in some patients with high levels of gamma globulin.[7, 26, 55] If this be true, then similar inhibition of endogenous insulin action could play a rôle in the undue hyperglycemia noted in glucose tolerance tests and perhaps in the diminished electrolyte changes following glucagon or epinephrine administration. In the last two, of course, a decreased availability of liver glycogen stores must also be a

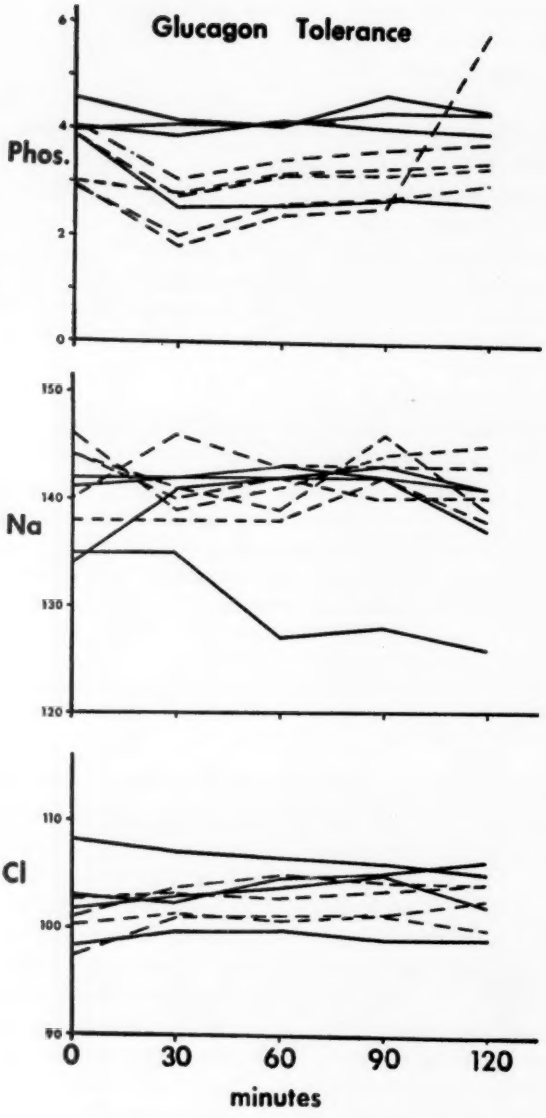

FIG. 4b. (See Fig. 4a for legend.)

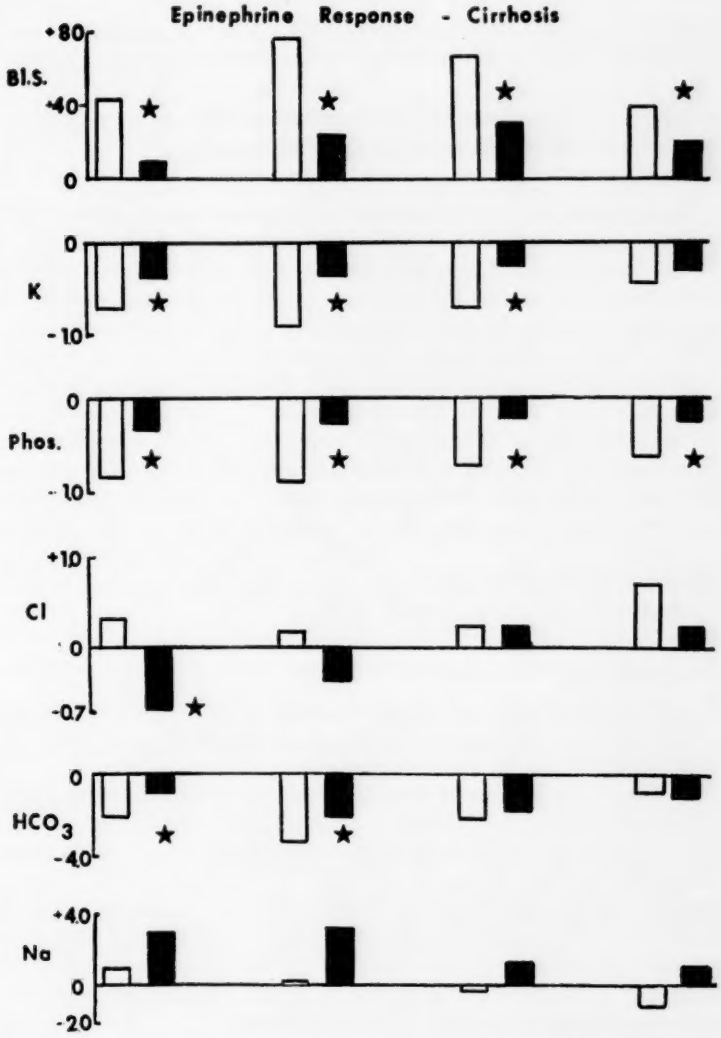

FIG. 5. Blood sugar and electrolyte responses in normals and in cirrhotics following epinephrine. The administration of epinephrine free of norepinephrine as indicated in the text produced less hyperglycemia, less hypokalemia, and less hypophosphatemia in 14 cirrhotics than in 11 healthy adults. In addition, the serum chloride decreased and the fall in serum total CO_2 content (HCO_3) was less.

factor. However, in view of the uncertainty whether glucagon and epinephrine enhance, inhibit, or have no effect upon the peripheral disposal of glucose,[6, 8, 17, 30–33, 58, 61, 62, 68] further discussion along this line is premature.

D. CANCELLATION OF ENHANCED ANAEROBIC GLYCOLYSIS AND OF INSULIN INHIBITION IN CIRRHOTICS GIVEN GLUCOSE AND INSULIN IN COMBINATION

The virtually equivalent responses of the blood sugar of the healthy control subjects and of the cirrhotic patients to intravenous glucose and insulin in combination are compatible with three hypotheses: (i) that under these circumstances the limited ability of the cirrhotic to store glucose as glycogen is enhanced, (ii) that glucose is removed by deposition as extrahepatic tissue glycogen or, (iii) that under these circumstances anaerobic glycolysis occurs in the cirrhotic at a rate which is increased sufficiently to make the over-all disposal of the carbohydrate load comparable in the two. There is no support in the serum inorganic phosphorus data for the last hypothesis (Fig. 3). From our limited observations it would appear, therefore, that when glucose and insulin are given to a cirrhotic in combination one does not see either the enhanced anaerobic glycolysis which follows glucose alone, nor the partial cancellation of insulin action evident after insulin per se. It may be that under these circumstances the glucose load is deviated to hepatic or extrahepatic glycogen.

SUMMARY AND CONCLUSIONS

1. Studies of blood sugar changes in cirrhotics given glucose, glucagon, or epinephrine show relative hyper- or hypoglycemia compatible with diminished ability to lay down or release liver glycogen.

2. The greater decrease in serum inorganic phosphorus following glucose infusion to patients with cirrhosis indicates that anaerobic glycolysis is enhanced even though the over-all glucose disappearance rate is less than in healthy control subjects.

3. Insulin administered intravenously has less of a hypoglycemic action in cirrhotics. This cannot be explained by diminished stores of liver glycogen since this would only tend to accentuate the hypoglycemic effect. The hypothesis is therefore advanced that in cirrhotic patients there occurs a partial concellation of the action of exogenous insulin.

4. In the cirrhotic the combined administration of glucose and insulin eliminates both the enhanced anaerobic glycolysis following glucose alone and the lesser hypoglycemia observed with insulin per se. Glucose disposal rates are of the same order of magnitude as in the healthy adult.

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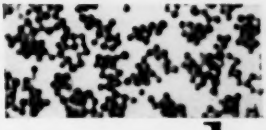
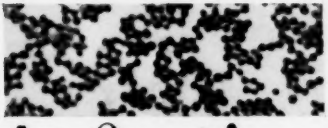
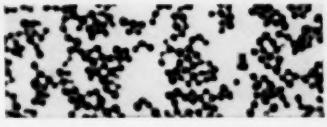
when sensitivity   testing reveals infection due to resistant staphylococci 

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